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PTOMAÏNES, LEUCOMAÏNES,

AND

BACTERIAL PROTEIDS:

OR

THE CHEMICAL FACTORS IN THE CAUSATION OF DISEASE.

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BY
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OF MICHIGAN.

SECOND EDITION, REVISED AND ENLARGED.

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study has certainly become one of great interest to all scientific students of medicine.

In the preparation of the present edition we have endeavored to utilize the latest and best information, and we can only express our thanks for the encouragement which we have received from so many sources and hope that the present effort will justify no censure.

UNIVERSITY OF MICHIGAN, SEPTEMBER, 1891.

PREFACE TO FIRST EDITION.

WITHIN the past ten years much has been said and written concerning the basic substances formed during the putrefaction of organic matter, and those which are produced by the normal tissue-changes in the living organism. Many investigators have given their whole time and attention to the study of these substances, and important discoveries have been made and much light has been thrown upon what have heretofore been considered problems in medical science. To collect, arrange, and systematize the facts concerning ptomaïnes and leucomaïnes has been our first object. Although many short essays, some of them of great value, have been written with the above-mentioned object in view, the present work may be regarded as the first attempt to make this collation embrace everything of importance on this subject. In endeavoring to accomplish this object we have met with many difficulties. The original reports of the various investigators are scattered through the pages of medical and scientific journals, transactions of societies, monographs, government reports, etc. However, with few exceptions we have been able to obtain the original reports, and we think that we have included everything of importance published up to the present year (1888).

To the physician the facts which have been made known concerning the putrefactive and physiological alkaloids must be of great value, and if this little work furnishes the means by which members of the profession may become better acquainted with the nature of those poisons which are introduced from without, and those which are generated within the body of man, the object of its authors will be accomplished.

UNIVERSITY OF MICHIGAN, JULY, 1888.

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PTOMAÏNES, LEUCOMAÏNES, AND BACTERIAL PROTEIDS.

INTRODUCTION.

It is customary to divide bacteria into the parasitic and the saprophytic. The obligate parasite can live only on living matter; the obligate saprophyte can live only on dead matter. Since all attempts to grow the bacilli of syphilis and leprosy on artificial media have failed, they are probably obligate parasites. True parasitic germs do not prove speedily fatal to their hosts, because their continued existence depends upon the continued existence of their host, or on their transference to another host. Leaving out of consideration the obligate bacterial parasites, about which very little is known at best, the above classification becomes of but little importance to us in a study of the causal relation of germs to disease, because a given bacterium may grow and multiply in one part of the body, while it is unable to do so in another; or it may thrive in one species of animal, while it finds the conditions unfavorable in another species; or similar differences may exist in individual members of the same species. Thus, the white rat is ordinarily and naturally immune against the bacillus of anthrax, but if the rat be exhausted by being kept on a small treadmill for some hours it becomes susceptible to anthrax. Recognizing these facts, we propose that bacteria be divided into the toxicogenic and the non-toxicogenic. Since we know of no infectious disease in which poisons are not formed, the toxicogenic germs only are of interest to us.

In the study of these we must not only ascertain the nature of the poisons which they produce, but must know the conditions under which they can multiply and elaborate these poisons. To these points the following pages, in so far as they treat of the infectious diseases, will be devoted.

However, all diseases are not infectious; all poisons formed within the body do not owe their existence to bacteria. Some originate in the altered metabolism of the various tissues, and these will be discussed under the autogenous diseases.

CHAPTER I.

DEFINITION AND CLASSIFICATION OF THE BACTERIAL POISONS.

Ptomaïnes.—An exact classification of the chemical factors in the causation of the infectious diseases can probably not be made at present. We know of two chemically distinct classes, one of which contains substances which combine with acids, forming chemical salts, and which in this respect at least correspond with the inorganic and vegetable bases. The members of this class are designated as ptomaïnes, a name suggested by the Italian toxicologist, Selmi, and derived from the Greek word πτωμα, meaning a cadaver. A ptomaïne may be defined as a chemical compound which is basic in character and which is formed by the action of bacteria on organic matter. On account of their basic properties, in which they resemble the vegetable alkaloids, ptomaïnes may be called putrefactive alkaloids. They have also been called animal alkaloids, but this is a misnomer, because, in the first place, some of them are formed in the putrefaction of vegetable matter; and, in the second place, the term "animal alkaloid" is more properly restricted to the leucomaïnes—those basic substances which result from tissue metabolism in the body. While some of the ptomaïnes are highly poisonous, this is not an essential property, and others are wholly inert. Indeed, the greater number of those which have been isolated up to the present time do not, when employed in single doses, produce any apparently harmful effects. Brieger restricts the term ptomaïne to the non-poisonous basic products, and designates the poisonous ones as "toxines." This is a classification, however, which seems to be of questionable utility. It is not always easy to say just what bodies are poisonous and what are not. The poisonous action of a substance depends upon the conditions under which, and the time during which, it is administered. Thirty grains of quinine may be taken by a healthy man during twenty-four hours without any appreciably ill effect, yet few of us would be willing to admit that the administration of this amount daily for three months would be wise or altogether free from injury. In the same manner the administration of a given quantity of a putrefactive alkaloid to a dog or guinea-pig in a single dose may do no harm, while the daily production of the same substance in the intestine of a man and its absorption continued through weeks and possibly months may be of marked detriment to the health. We do not as yet know enough about the physiological or toxicological action of the putrefactive alkaloids to render the classification proposed by **Brieger** worthy of general adoption.

All ptomaïnes contain nitrogen as an essential part of their basic character. In this they resemble the vegetable alkaloids. Some of them contain oxygen, while others do not. The latter correspond to the volatile vegetable alkaloids, nicotine and coniine, and the former correspond to the fixed alkaloids.

Since all putrefaction is due to the action of bacteria, it follows that all ptomaïnes result from the growth of these microörganisms. The kind of ptomaïne formed will depend upon the individual bacterium engaged in its production, the nature of the material being acted upon, and the conditions under which the putrefaction goes on, such as the temperature, amount of oxygen present, and the duration of the process.

Brieger found that, although the Eberth bacillus grew well in solutions of peptone, it did not produce any ptomaïne; while from cultures of the same bacillus in beef-tea he obtained a poisonous alkaloid. **Fitz** found that whilst the bacillus butyricus produces by its action on carbohydrates butyric acid, in glycerin it produces propylic alcohol, and **Morin** has found amyl alcohol among the products of this germ. **Brown** has shown that while the mycoderma aceti converts ethylic alcohol into acetic acid, it converts propylic alcohol into propionic acid, and is without effect upon methylic alcohol, primary isobutylic alcohol, and amylic alcohol. Some bacteria will not multiply below a given temperature. Thus, the bacillus butyricus will not grow at a temperature below 24°.[1] The lower temperature does not destroy the organism, but it lies dormant until the conditions are more favorable for its growth. PASTEUR divided the bacteria into two classes—the aërobic and the anaërobic. As the name implies, the former grow and thrive in the presence of air, while the latter find their conditions of life improved by the exclusion of air. Therefore, different ptomaïnes will be formed in decomposing matter freely exposed to the air, and in that which is buried beneath the soil or from which the air is largely excluded. Even when the same ferment is present the products of the putrefaction will vary, within certain limits, according to the extent to which the putrefying material is supplied with air. The kind of ptomaïne found in a given putrid substance will depend also upon the stage of the putrefaction. Ptomaïnes are transition products in the process of putrefaction. They are temporary forms through which matter passes while it is being transformed, by the activity of bacterial life, from the organic to the inorganic state. Complex organic substances, as muscle and brain, are broken up into less complex molecules, and so the process of chemical division goes on until the simple and well-known final products, carbonic acid gas, ammonia, and water, result; but the variety of combinations into which an individual atom of carbon may enter during this long series of changes is almost unlimited, and with each change in combination there is more or less change in nature. In one combination the atom of carbon may exist as a constituent of a highly poisonous substance, while the next combination into which it enters may be wholly inert.

It was formerly supposed that putrefaction was simply oxidation, but the researches of PASTEUR and others have demonstrated the fact that countless myriads of minute organisms are engaged constantly in transforming matter from the organic to the inorganic form. Lock up the bit of flesh so that these little workers cannot reach it, and it will remain unchanged indefinitely.

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[1] All temperatures given in this work are Centigrade, unless otherwise specified.

It may be asked if any of the changes occurring during putrefaction are to be regarded as purely chemical. Without doubt, many of the secondary products of putrefaction arise from reactions between antecedent and more complex products or by the action of oxygen, water, and reducing agents upon primary products. Ptomaïnes formed in this way may be regarded as the indirect results of bacterial life.

BACTERIAL PROTEIDS.—These substances have been known for so short a time and are at present so imperfectly known that many difficulties arise in discussing them. In the first place, we may divide the bacterial proteids into two classes: (1) those which constitute an integral part of the bacterial cells, and (2) those which have not been assimilated by the cells, but which have been formed by the fermentative or cleavage action of the bacteria on the proteid bodies in which they are growing. Even this classification is of questionable value. We allow bacteria to grow for a number of days in a nutrient solution. We then separate the soluble constituents from the formed cells by filtration through porous tile; we wash the latter and then study their proteid contents, which constitute the first class, as given above; but the filtrate contains, or may contain, any one or more of the following proteid bodies: (1) Those portions of the proteid substances which were used in the preparation of the nutrient solution and which have escaped the action of the bateria; (2) proteids which have been at one time integral parts of the cells, but which have passed into solution on the death and dissolution of the bacteria; and (3) proteids which have been formed by the fermentative action of the bacteria, or those which are defined as constituting the second class, as given above. We know at present of no means by which one of these proteids can with certainty be isolated from the others. However, the above classification is a convenient one, and with a clear understanding that it is not free from criticism we may employ it until a more thorough and scientific study of these bodies has been made.

The difficulty in discussing these substances lies not only in the classification, but in the name which shall be employed to designate them. **Brieger** and **Fränkel** have proposed the term "toxalbumins;" but, while it is true that some belong to the albumins, others are more truly albumoses; others are most closely related to the peptones; and still others differ in some important respects from all of these. In view of the above facts, we have decided upon the term "bacterial proteids" to designate those formed by the fermentative action of germs, while those which constitute an integral part of the cell will be known as "the bacterial cellular proteids."

The Bacterial Cellular Proteids.—**Nencki** first prepared one of these substances from putrefactive bacteria. These were obtained by decantation, freed from fat with ether, dissolved in fifty parts of a potash solution of 0.5 per cent., heated for some hours at 100° and filtered. The filtrate was acidified with dilute hydrochloric acid and precipitated by the addition of rock salt. The precipitate was washed with a saturated salt solution, dried at 100°, and washed free from salt with water. **Nencki** designates this substance as "mycoprotein," and finds that it has the formula, $C_{25}H_{42}N_6O_9$. Freshly precipitated mycoprotein forms in amorphous flakes, which are soluble in water, alkalies, and acids. The aqueous solution is acid in reaction. After being dried at 100° it is no longer wholly soluble in water. **Nencki** found that it is not precipitated from aqueous solution by alcohol, but by picric acid, tannic acid, and mercuric chloride; that it does not give the xanthoproteid, but does give the Millon and the biuret reactions. According to **Schäffer** it is changed by acids into peptone, and on being fused with five parts of potash it breaks up into ammonia, amylamin, phenol (0.15 per cent. of its weight), valerianic acid (38 per cent.), leucine, and traces of indol and skatol. A proteid obtained from the yeast plant has the formula, $C_{12}H_{21}N_3O_3$.

The purified pyogenetic agent obtained from the pneumonia bacillus of **Friedländer** was found by **Büchner** to give the following reactions: It is soluble in water and the concentrated mineral acids, very soluble in dilute alkalies, from which it is precipitated on the addition of an acid. From its aqueous solution, it is not precipitated by heat, nor by saturation with sodium chloride, but is precipitated by magnesium sulphate, copper sulphate, platinum chloride, gold chloride, lead salts, picric acid, tannic acid, and absolute alcohol. It gives the xanthoproteid, Millon, and biuret reactions.

The Bacterial Proteids.—**Brieger** and **Fränkel** obtained the proteid poison of diphtheria by precipitating the filtrate from a Chamberland filter after concentration to one-third its volume at 30°, with absolute alcohol after feebly acidifying with acetic acid. The precipitate was purified by repeated solution in water and reprecipitation with alcohol. Dried in a vacuum at 40°, it forms a snow-white, amorphous, very light mass. From its aqueous solution it is not precipitated by heat or dilute nitric acid, singly or combined, nor by sodium sulphate, sodium chloride, magnesium sulphate, or lead salts. It is precipitated by carbonic acid (to saturation), concentrated mineral acids, potassium ferrocyanide and acetic acid, phenol, organic acids (soluble in excess), copper sulphate, silver nitrate, and mercuric chloride. The so-called alkaloidal reagents, phosphomolybdic acid, potassio-mecuric iodide, potassio-bismuthic iodide, platinum chloride, gold chloride, and picric acid also cause precipitation. The xanthoproteid, Millon, and biuret reactions give positive results. An ultimate analysis furnishes the following figures computed from the ash-free substance: C 45.35, H 7.13, N 16.33, S 1.39, O 29.80. From these facts **Brieger** and **Fränkel** conclude that this substance is allied to serum-albumin. Their bouillon cultures contain serum-albumin, and they suppose that the bacteria convert this into the poison by causing a rearrangement in the atoms; but the same poison was formed when nutrient solutions containing no proteid save peptone were employed. In this case they suppose that the bacteria reconvert the peptone into an albumin.

The poisonous proteids obtained by **Brieger** and **Fränkel** from cultures of the Eberth germ, the comma bacillus, and the staphylococcus aureus are practically insoluble in water, and more nearly related to the globulins than the albumins, although they differ from the former in their tardy and difficult solubility in dilute solutions of sodium chloride.

The poisonous proteids isolated by **Vaughan** from cultures of two species of toxicogenic germs found in drinking water, supposed to be the cause of typhoid fever, are soluble in water, from which they are not precipitated by boiling, or by concentrated nitric acid, or by both. Potassium ferrocyanide and acetic acid, sodium sulphate, magnesium sulphate, and carbonic acid also fail to precipitate them. They are precipitated by the general alkaloidal reagents, and respond to the xanthoproteid, Millon, and biuret tests. They are precipitated by ammonium sulphate when added to saturation, and for this reason cannot be classed among the peptones. Neither benzoyl chloride nor phenyl-hydrazin chloride precipitate them. Their poisonous properties are destroyed by prolonged boiling or by being heated to 80° for some hours, though they remain active after an exposure of ten minutes to the last mentioned temperature.

Of the three bacterial proteids obtained by the same experimenter from the bacilli x, a and A of Booker's list of summer diarrhœa germs, the first two are soluble in water, while the other is not. So far as their behavior with precipitating agents is concerned, the first two agree with the proteids of the water germs.

Tizzoni and **Cattani** find that the proteid of cultures of their tetanus germ is rendered inert by precipitation with absolute alcohol. It is obtained by saturation with ammonium sulphate, and the removal of the salt by dialysis.

Further description of the individual proteids will be given in subsequent chapters.

CHAPTER II.

HISTORICAL SKETCH OF THE BACTERIAL POISONS.

IT must have been known to primitive man that the eating of putrid flesh was liable to affect the health more or less seriously; and when he began his endeavors to preserve his food for further use, instances of poisoning from putrefaction must have multiplied. However, the distinguished physiologist, ALBERT VON HALLER, seems to have been the first to make any scientific experiments concerning the effects of putrid matter upon animals. He injected aqueous extracts of putrid material into the veins and found that death resulted. Later in the eighteenth century MORAND gave an account of the symptoms induced by eating poisonous meat. In the early part of the present century (1808 to 1814) GASPARD carried on similar experiments. He use as material the putrid flesh of both carnivorous and herbivorous animals. With these he induced marked nervous disturbances, as stiffness of the limbs, opisthotonos, and tetanus. GASPARD concluded from the symptoms that the poisonous effects were not due to carbonic acid gas or hydrogen sulphide, but thought it possible that ammonia might have part in their production. In 1820 KERNER published his first essay on poisonous sausage, which was followed by a second in 1822. At first he thought that the poisonous properties were due to a fatty acid, similar to the sebacic of THENARD, and which originated during putrefaction. Later he modified these views, and believed the poison to be a compound consisting of the sebacic acid and a volatile principle. This may be regarded as the first suggestion as to the probability of the development of a poisonous substance with basic properties in decomposing matter. In 1822, DUPRÉ observed a peculiar disease among the soldiers under his care, who, during the warm and dry summer of that year, were compelled to drink very foul water. Later MAGENDIE, induced by the investigations of GASPARD and the observations of DUPRÉ, made many experiments, in which dogs and other animals were confined over vessels containing putrid animal matter and compelled constantly to breathe the emanations therefrom. The effects varied markedly with the species of animal and the nature of the putrid material, but in some instances symptoms were induced which resembled closely those of typhoid fever in man. LEURET directed his attention to the chemical changes produced in blood by putrefaction, but accomplished nothing of special value. DUPUY injected putrid material into the jugular vein of a horse, and with TROUSSEAU studied alterations produced in the blood by these injections.

During the third decade of the present century there were many investigators in addition to those mentioned above, who endeavored to ascertain the active agent in poisonous foods. DANN, WEISS, BÜCHNER, SCHUMANN, CADET DE GASSICOURT, and ORFILA studied poisonous sausage, but made no advance upon the work done by KERNER. HENNEMAN, HÜNNEFELD, WESTRUMB, and SERTÜRNER made contributions concerning poisonous cheese, but all believed the caseic acid of KERNER to be the poisonous principle.

In 1850 SCHMIDT, of Dorpat, made some investigations on the decomposition products and volatile substances found in cholera stools; and, two years later, MEYER, of Berlin, injected the blood and stools of cholera patients into lower animals. In 1853 STICH made an important contribution on the effects of acute poisoning with putrid material. He ascertained that, when given in sufficient quantity, putrid matter produces an intestinal catarrh, with choleraic stools. Nervous symptoms, trembling, unsteady gait, and, finally, convulsions were also observed. STICH made careful post-mortem examinations, and was unable to find any characteristic or important lesions. Theoretically, he concluded that the putrid material contained a ferment which produced rapid decomposition of the blood.

In 1856 PANUM published a most important contribution to the knowledge of the nature of the poison present in putrid flesh. He first demonstrated positively the chemical character of the poison, inasmuch as he showed that the aqueous extract of the putrid material retained its poisonous properties after treatment which would insure the destruction of all organisms. His conclusions were as follows:

(1) "The putrid poison contained in the decomposed flesh of the dog, and which is obtained by extraction with distilled water and repeated filtration, is not volatile, but fixed. It does not pass over on distillation, but remains in the retort.

(2) "The putrid poison is not destroyed by boiling, nor by evaporation. It preserves its poisonous properties even after the boiling has been continued for eleven hours, and after the evaporation has been carried to complete desiccation at 100°.

(3) "The putrid poison is insoluble in absolute alcohol, but is soluble in water, and is contained in the aqueous extract which is formed by treating with distilled water the putrid material which has previously been dried by heat and washed with alcohol.

(4) "The albuminoid substances which frequently are found in putrid fluids are not in themselves poisonous only so far as they contain the putrid poison fixed and condensed upon their surfaces, from which it can be removed by repeated and careful washing.

(5) "The intensity of the putrid poison is comparable to that of the venom of serpents, of curare, and of certain vegetable alkaloids, inasmuch as 0.012 of a gramme of the poison, obtained by extracting with distilled water putrid material which had been previously boiled for a long time, dried at 100°, and submitted to the action of absolute alcohol, was sufficient almost to kill a small dog."

PANUM made intravenous injections with this poison, and with ammonium carbonate, ammonium butyrate, ammonium valerianate, tyrosine, and leucine, and found that the symptoms induced by the putrid poison differed from those caused by the other agents. Moreover, he found the symptoms to differ from those of typhoid fever, cholera, pyæmia, anthrax, and sausage poisoning. He was also in doubt as to whether the poison acted directly upon the nervous system, or whether it acted as a ferment upon the blood, causing decomposition, the products of which affected the nerve-centres; but he was sure that it could not correspond to the ordinary ferments, inasmuch as it was not decomposed by prolonged boiling nor by treatment with absolute alcohol. Certainly, the putrid poison could not consist of a living organism.

The symptoms observed by PANUM varied greatly with the quantity of the poison used and the strength of the animal. After the intravenous injection of large doses, death followed in a very short time. In these cases there were violent cramps, and involuntary evacuations of the urine and feces; the respirations were labored, the pallor was marked, sometimes followed by cyanosis, the pulse feeble, the pupils widely dilated, and the eyes projecting. In these cases the autopsy did not reveal any lesion, save that the blood was dark, imperfectly coagulated and slightly infiltrated through the tissue. Post-mortem putrefaction came on with extraordinary rapidity.

When smaller doses or more vigorous animals were used, the symptoms did not appear before from a quarter of an hour to two hours, and sometimes even later. In these cases the symptoms were less violent, and the animal generally recovered. In all instances, however, the disturbances were more or less marked.

In addition to the "putrid poison," PANUM obtained a narcotic substance, the two being separated by the solubility of the narcotic in alcohol. The alcoholic extract was evaporated to dryness, the residue dissolved in water and injected into the jugular vein of a dog. The animal fell into a deep sleep, which remained unbroken for twenty-four hours, when it awoke apparently in perfect health.

PANUM'S first contributions, which were published in Danish, did not attract the attention which they deserved, until after the lapse of several years. Now, however, their importance is fully appreciated, and the distinguished investigator lived to receive the credit and honor due him.

WEBER, in 1864, and HEMMER and SCHWENNINGER in 1866, confirmed the results obtained by PANUM; and SCHWENNINGER announced that in the various stages of putrefaction different products are formed, and that these vary in their effects upon animals. In 1866, BENCE JONES and DUPRÉ obtained from the liver a substance which in solutions of dilute sulphuric acid gives the blue fluorescence observed in similar solutions of quinine. To this substance they gave the name "animal chinoidine." Subsequently, the same investigators found this substance in all organs and tissues of the body, but most abundantly in the nerves. Its feebly acid solutions give precipitates with iodine, potassio-mercuric iodide, phospho-molybdic acid, gold chloride, and platinum chloride. From three pounds of sheep's liver, they obtained three grammes of a solution in which, after slight acidulation with sulphuric acid, the intensity of the fluorescence was about the same as that of a similarly acidulated solution of quinine sulphate which contained 0.2 gramme of quinine per litre. Still later, this base was obtained by MARINO-ZUCO.

In 1868, BERGMANN and SCHMIEDEBERG separated, first from putrid yeast, and subsequently from decomposed blood, in the form of a sulphate, a poisonous substance which they named sepsine. The sulphate of sepsine forms in needle-shaped crystals. Small doses (0.01 gramme) of this substance were dissolved in water and injected into the veins of two dogs. In a short time it produced vomiting, and later diarrhœa, which, in one of the animals, after a time, became bloody. Post-mortem examination showed, in the stomach and intestines, bloody ecchymoses. It was now believed that the "putrid poison" of PANUM had been isolated, and that it was identical with sepsine, but further investigations showed that this was not true. There are marked differences in their effects upon animals, and sepsine has not been found to be generally present in putrid material. It is only rarely found in blood, and the closest search has failed to show its presence in pus. BERGMANN, following the same method which he had used in extracting this poison from yeast, has been unable to obtain it from other putrid material. Moreover, he was not always successful in obtaining the poison from yeast. Sepsine was not obtained in quantity sufficient to serve for an ultimate analysis, hence, its composition remains unknown.

In 1869 **Zülzer** and **Sonnenschein** prepared from decomposed meat extracts a nitrogenous base, which in its chemical reactions and physiological effects resembled atropine and hyoscyamine. When injected under the skin of animals it produced dilatation of the pupils, paralysis of the muscles of the intestines, and acceleration of the heartbeat; but it is uncertain and inconstant in its action. This probably results from rapid decomposition taking place in it, or to variations in its composition at different stages of putrefaction. This substance has also been obtained from the bodies of those who have died from typhoid fever, and it may be possible that the belladonna-like delirium which frequently characterizes the later stages of this disease is due to the ante-mortem generation of this poison within the body.

Since 1870 many chemists have been engaged in making investigations on the products of putrefaction. We can only mention a few names at present, while others will be referred to subsequently in discussing the individual ptomaïnes.

First of all stands the Italian **Selmi**, who suggested the name ptomaïne, and whose researches furnished us with much information of value, and, what is probably of more importance, gave an impetus to the study of the chemistry of putrefaction, which has already been productive of much good and gives promise of much more in the future. **Selmi** showed that ptomaïnes could be obtained (1) by extracting acidified solutions of putrid material with ether; (2) by extracting alkaline solutions with ether; (3) by extracting alkaline solutions with chloroform; (4) by extracting with amylic alcohol; and (5) that there yet remained in the solutions of putrid matter ptomaïnes which were not extracted by any of the above-mentioned reagents. In this way he gave some idea of the great number of alkaloidal bodies which might be formed among the products of putrefaction, and the promising field thus discovered and outlined was soon occupied by a busy host of chemists. In the second place, he demonstrated the fact that many of the ptomaïnes give reactions similar to those given by the vegetable alkaloids. This led the toxicologist into investigations, the results of some of which we will ascertain further on.

SELMI, however, did not succeed in isolating completely a single putrefactive alkaloid. All his work was done with extracts. He remained ignorant, except in a general way, of the composition of these bodies. NENCKI, in 1876, made the first ultimate analysis and determined the first formula of a ptomaïne. This was an isomer of collodine, which will be described later.

RÖRSCH and FASSBENDER, in a case of suspected poisoning, obtained by the STAS-OTTO method a liquid which could be extracted from acid as well as alkaline solutions by ether, and which gave all the general alkaloidal reactions. They were unable to crystallize either extract by taking it up with alcohol and evaporating. The colorless aqueous solution was not at all bitter to the taste. The precipitate formed with phospho-molybdic acid dissolved on the application of heat, giving a green solution, which became blue on the addition of ammonia. They believed that this substance was derived from the liver, since fresh ox-liver, treated in the same manner, gave them an alkaloid which could be extracted with ether from acid as well as from alkaline solutions. GUNNING found this same alkaloid in liver-sausage from which poisoning had occurred. RÖRSCH and FASSBENDER state that while in some of its reactions this substance resembles digitaline, it is distinguished from this vegetable alkaloid by the failure of the ptomaïne to give the characteristic bitter taste.

SCHWANERT, whilst examining the decomposing intestines, liver, and spleen of a child which had died suddenly, perceived a peculiar odor and obtained by the STAS-OTTO method (ether extract from an alkaline solution) small quantities of a base, which was distinguished from nicotine and coniine by its greater volatility and its peculiar odor. He supposed that this substance was produced by decomposition, and, in order to ascertain the truth of his supposition, he took the organs of a cadaver that had lain for sixteen days at a temperature of 30° and was well decomposed. These were treated with tartaric acid and alcohol. The acid solution was first extracted with ether, and yielded no result; it was then rendered alkaline and extracted with ether. The latter extract gave, on evaporation, the same substance which he had found in the organs of the child. The residue was a yellowish oil, having an odor somewhat similar to propylamine. It was repulsive, but not bitter to the taste, and alkaline in reaction. On the addition of hydrochloric acid, it crystallized in white needles, which were freely soluble in water, but soluble with difficulty in alcohol. On the addition of ammonium hydrate to this crystalline substance, a white vapor of unpleasant odor was given off. The crystals dissolved in sulphuric acid, forming a solution which was at first colorless, but which gradually became dirty brownish-yellow, and grayish-brown on the application of heat. On being warmed with sodium molybdate, a splendid blue color, becoming gradually gray, was produced. Potassium bichromate and sulphuric acid gave a reddish-brown, then a grass-green color. Nitric acid gave a yellow color. A tartaric acid solution of the crystals produced, on the addition of platinum chloride, a dirty yellow precipitate of small six-sided stars, which contained 31.55 per cent. of platinum. Gold chloride gave a pale yellow, amorphous precipitate; mercuric chloride yielded white crystals; potassio-mercuric iodide a dirty-white precipitate; and potassio-cadmic iodide yielded no result. Tannic acid produced only a turbidity. Sodium phospho-molybdate gave a yellow, flocculent precipitate, which became blue on the addition of ammonium hydrate. This base has a slight reducing power, and in this it resembles a substance obtained by SELMI, but it differs from SELMI's extract inasmuch as it does not give a violet coloration on being warmed with sulphuric acid. In its amorphous character, its behavior to the general alkaloidal reagents, and its lack of bitter taste, it resembles the base obtained by **Rörsch** and **Fassbender**, but, unlike that alkaloid, it is extractable from alkaline solutions only.

Selmi, in commenting upon the base studied by **Rörsch** and **Fassbender**, **Schwanert**, and himself, believing that all were dealing with the same body, states that it does not contain phosphorus, and that it is separated with extreme difficulty from the vegetable alkaloids.

Liebermann, in examining the somewhat decomposed stomach and intestines in a case of suspected poisoning, found an alkaloidal body which was unlike that studied by the chemists mentioned above, inasmuch as it was not volatile. The **Stas-Otto** method was employed. The ether extract from alkaline solution left, on evaporation, a brownish, resinous mass, which dissolved in water to a turbid solution, the cloudiness increasing on heating. This reaction agrees with coniine, but the odor differed from that of the vegetable alkaloid. The aqueous, strongly alkaline solution gave the following reactions:

(1) With tannic acid, a white precipitate.

(2) With potassium iodide, a yellowish-brown, turning to dark-brown precipitate.

(3) With chlorine water, a marked white cloudiness.

(4) With phospho-molybdic acid, a yellow precipitate.

(5) With potassio-mercuric iodide, a white precipitate.

(6) With mercuric chloride, a white cloudiness.

(7) With concentrated sulphuric acid, after a while, a reddish-violet coloration.

(8) With concentrated nitric acid, after evaporation, a yellowish spot.

These reactions exclude all vegetable alkaloids save coniine. The putrefactive alkaloid does not distil when heated on the oil-bath to 200°, while coniine distils at 135°. The former is with certainty distinguished from coniine by its non-poisonous properties.

This substance is extracted by ether from acid, as well as from alkaline solutions. The yellow, oily drops obtained after the evaporation of the ether are soluble in alcohol. The taste is slightly burning.

SELMI obtained from both putrefying and fresh intestines a substance which gave the general alkaloidal reactions with potassium iodide, gold chloride, platinum chloride, potassio-mercuric iodide, and phospho-molybdic acid. It has strong reducing power, and when warmed with sulphuric acid gives a violet coloration. These reactions are not due to leucine, tyrosine, creatine, or creatinine. This is the substance which, as has been stated, SELMI considered identical with that observed by RÖRSCH and FASSBENDER and SCHWANERT. The minor differences observed by the different chemists may have been due to the varying degrees of purity in which the substance was obtained by them.

From human bodies which had been dead from one to ten months, SELMI removed many alkaline bases. From an ether solution of a number of these, one was removed by treatment with carbonic acid gas. One base which was insoluble in ether, but readily soluble in amylic alcohol, was found to be a violent poison, producing in rabbits tetanus, marked dilatation of the pupils, paralysis, and death.

Parts of a human body preserved in alcohol were found by SELMI to yield an easily volatile, phosphorus-containing substance, which is soluble in ether and carbon disulphide, and gives a brown precipitate with silver nitrate. It is not the phosphide of hydrogen. A similar substance is produced by the slow decomposition of the yolks of eggs. With potassium hydrate it gives off ammonia and yields a substance having an intense coniine odor. It is volatile and reduces phosphomolybdic acid.

SELMI also obtained from decomposing egg-albumin a body, whose chloride forms in needles, and which has a curare-like action on frogs. From one arsenical body which had been buried for fourteen days, he obtained, by extracting from an alkaline (made alkaline with baryta) solution with ether, a substance which formed in needles and which gave crystalline salts with acids. With sulphuric acid it gave a red color; with iodic acid and sulphuric acid it liberated free iodine and gave a violet coloration; with nitric acid it gave a beautiful yellow, which deepened on the addition of caustic potash. Platinum chloride gave no precipitate save in highly concentrated solutions. From a second arsenical body, SELMI obtained by the same method a substance which gave, with tannic acid, a white precipitate; with iodine in hydriodic acid a kermes-brown; with gold chloride a yellow, which was soon reduced; with mercuric chloride a white; with picric acid, a yellow, which gradually formed in crystalline tablets. This substance did not contain any arsenic, but was highly poisonous. From the stomach of a hog, which had been preserved in a solution of arsenious acid, SELMI separated an arsenical organic base. The fluid was distilled in a current of hydrogen. The distillate, which was found to be strongly alkaline, was neutralized with hydrochloric acid and evaporated to dryness, when cross-shaped crystals, giving an odor similar to that of trimethylamine, were obtained. This substance was found by CIACCIA to be highly poisonous, producing strychnia-like symptoms. With iodine in hydriodic acid it is said to give a gray, crystalline precipitate.

From the liquid which remained in the retort, a non-volatile arsenical ptomaïne was extracted with ether. An aqueous solution of this gave with tannic acid a slowly forming, yellowish precipitate, and similarly colored precipitates with iodine in hydriodic acid, platinum chloride, auric chloride, mercuric chloride, potassio-mercuric iodide, potassio-bismuthic iodide, picric acid, and potassium bichromate. The physiological action of this substance as demonstrated on frogs was unlike that of the arsines, but consisted of torpor and paralysis.

MORIGGIA and BATTISTINI experimented with alkaloids, obtained from decomposing bodies, upon guinea-pigs and frogs, but did not attempt their isolation because of the rapid decomposition which they undergo when exposed to the air and by which they lose their poisonous properties. These alkaloids they found to be easily soluble in amylic alcohol, less soluble in ether.

In 1871 LOMBROSO showed that the extract from mouldy corn-meal produced tetanic convulsions in animals. This threw some light upon the cases of sporadic illness which had long been known to occur among the peasants of Lombardy, who eat fermented and mouldy corn-meal. In 1876 BRUGNATELLI and ZENONI obtained by the STAS-OTTO method from this mouldy meal an alkaloidal substance which was white, non-crystalline, unstable, and insoluble in water, but readily soluble in alcohol and ether. With sulphuric acid and bichromate of potassium it yields a color reaction very similar to that of strychnine.

The action of the ether extracts from decomposed brain resembles that of curare, but is less marked and more transitory. The beats of the frog's heart were decreased in number and strengthened in force; the nerves and the muscles lost their irritability, and the animal passed into a condition of complete torpor. The pupils were dilated. GUARESCHI and MOSSO, using the STAS-OTTO method, obtained from human brains which had been allowed to decompose at a temperature of from 10° to 15° for from one to two months, both volatile and non-volatile bases. Among the former only ammonia and trimethylamine were in sufficient quantity for identification. With these, however, were minute traces of ptomaïnes.

They obtained non-volatile bases from both acid and alkaline solutions. From the former they separated a substance which gave precipitates with gold chloride, phospho-tungstic acid, phospho-molybdic acid, MAYER's reagent, palladium chloride, picric acid, iodine in potassium iodide, and slightly with tannic acid. This substance was not precipitated with platinum or mercury.

From the alkaline extract there was obtained a substance which in dilute hydrochloric acid solutions gave with gold chloride a heavy yellow precipitate with reduction, also precipitates with phospho-molybdic acid, platinum chloride, MAYER's reagent, picric acid, phospho-tungstic acid, MARMÉ's reagent, iodine in potassium iodide, tannin, bichromate of potassium, palladium chloride, and mercuric chloride. It reduces ferric salts. From decomposed fibrin the same investigators obtained one well-defined ptomaïne. Analyses of the platinum compound of this substance gave the formula $C_{10}H_{15}N$. This substance will be discussed in a future chapter.

From fresh brain substance they separated ammonia, trimethylamine, and an undetermined base. These, however, are not to be regarded as products of putrefaction, but as resulting from the action of the reagents upon the brain substance. The trimethylamine probably arises from the splitting up of lecithin, while the undetermined base is most likely choline, which also results from the breaking up of the lecithin molecule.

They also show that when **Dragendorff's** method is used basic substances can be obtained from fresh meat, and these are shown to be produced by the action of the sulphuric acid on the flesh.

To **Brieger**, of Berlin, is due the credit of isolating and determining the composition of a number of ptomaïnes. From putrid flesh he obtained neuridine, $C_5H_{14}N_2$, and neurine, $C_5H_{13}NO$. The former is inert, while the latter is poisonous. From decomposed fish he separated a poisonous base, $C_2H_4(NH_2)_2$, which is an isomeride of ethylenediamine, muscarine, $C_5H_{15}NO_3$, and an inert substance, $C_7H_{17}NO_2$, gadinine. Rotten cheese yielded neuridine and trimethylamine. Decomposed glue gave neuridine, dimethylamine, and a muscarine-like base. In the cadaver, he has found in different stages of decomposition, choline, neuridine, trimethylamine, cadaverine, $C_5H_{14}N_2$, putrescine, $C_4H_{12}N_2$, and saprine, $C_5H_{16}N_2$. These are all inert. After fourteen days of decomposition he found a poisonous substance, mydaleine. From a cadaver which had been kept at from $-9°$ to $+5°$ for four months, **Brieger** obtained mydine, $C_8H_{11}NO$, the poisonous substance mydatoxine, $C_6H_{13}NO_2$, also the poison methyl-guanidine. From poisonous mussel he separated mytilotoxine, $C_6H_{15}NO_2$. From pure cultures of the typhoid bacillus of **Koch** and **Eberth**, **Brieger** obtained a poison, typhotoxine, and, from like cultures of the tetanus germ of **Rosenbach**, tetanine. All of these bases will be discussed in detail in a subsequent chapter.

Gautier and Etard have also isolated ptomaïnes which will be described later.

In 1885, Vaughan succeeded in isolating an active agent from poisonous cheese, to which he gave the name tyrotoxicon. This discovery has been confirmed by Newton, Wallace, Schäffer, Stanton, Firth, Ladd, Wolff, Kimura, Davis, and Kinnicutt.

Nicati and Rietsch, Koch, and others, have shown the presence of a poisonous substance in cultures of the cholera bacillus. Salmon and Smith have done the same with cultures of the swine-plague germ; Hoffa, with those of the anthrax bacillus; and Brieger with those of the tetanus germ.

In 1888, Christmas obtained from cultures of the staphylococcus pyogenes aureus a proteid which, when injected into the anterior chamber of the eye or under the skin, causes suppuration.

In 1889, Hankin isolated from cultures of the bacillus anthracis a poisonous albumose, which, when employed in large doses, proves fatal, and in small doses gives immunity.

In 1888, Roux and Yersin showed that the chemical poison of Löffler's diphtheria bacillus is a proteid body which they believed to be of the nature of a ferment. In 1890, this work was continued by Brieger and Fränkel in their memorable contribution on bacterial poisons, in which they detail the methods by which they isolate their "toxalbumins" from cultures of the Löffler bacillus, the anthrax bacillus, Eberth's germ, the cholera vibrio, and the staphylococcus pyogenes aureus. Martin made a more detailed study of the albumoses of anthrax. Vaughan reported poisonous proteids in cultures of two toxicogenic germs found in drinking-water, also in cultures of three of Booker's summer diarrhœa germs and in poisonous cheese. Novy and Schweinitz found both basic and proteid poisons in cultures of the swine-plague bacillus.

Many other contributions have been made, many of which will be mentioned in subsequent chapters.

CHAPTER III.

FOODS CONTAINING BACTERIAL POISONS.

POISONOUS MUSSELS.—Judging from the symptoms produced, there seem to be three different kinds of poisonous mussel. In one class, the symptoms resemble those of a true gastro-intestinal irritant. **FODERE** reports the case of a sailor, who, after eating a large dish of mussels, suffered from nausea, vomiting, pain in the stomach, tenesmus, and rapid pulse. After death, which occurred within two days, the stomach and intestines were found inflamed and filled with a tenacious mucus. **COMBE** and others also report cases of the choleraic form of poisoning from mussel.

However, the symptoms which most frequently manifest themselves after the eating of poisonous mussels are more purely nervous. A sensation of heat and itching appears usually in the eyelids, and soon involves the whole face, and perhaps a large portion of the body. An eruption, usually called nettle-rash, though it may be papular or vesicular, covers the parts. The itching is most annoying, and may be accompanied by marked swelling. There follows a distressing asthmatic breathing, which is relieved by ether. In some cases reported by **MOHRING**, dyspnœa preceded the eruption, the patients became insensible, the face livid, and convulsive movements of the extremities were noticed. **BURROW** reports similar cases with delirium, convulsions, coma, and death within three days.

In a third class of cases, there may be a kind of intoxication resembling somewhat that of alcohol, then paralysis, coma, and death.

In 1827, **COMBE** observed thirty persons poisoned, two of them fatally, with mussels. He describes the symptoms as follows: "None, so far as I know, complained of anything peculiar in the smell or taste of the animals, and none suffered immediately after taking them. In general, an hour or two elapsed, sometimes more; and the bad effects consisted rather in uneasy feelings and debility than in any distress referable to the stomach. Some children suffered from eating only two or three; and it will be remembered that Robertson, a young and healthy man, only took five or six. In two or three hours they complained of a slight tension at the stomach. One or two had cardialgia, nausea, and vomiting; but these were not general or lasting symptoms. They then complained of a prickly feeling in their hands, heat and constriction of the mouth and throat; difficulty of swallowing and speaking freely; numbness about the mouth, gradually extending to the arms, with great debility of the limbs. The degree of muscular debility varied a good deal, but was an invariable symptom. In some it merely prevented them from walking firmly, but in most of them it amounted to perfect inability to stand. While in bed they could move their limbs with tolerable freedom, but on being raised to the perpendicular posture they felt their limbs sink under them. Some complained of a bad, coppery taste in the mouth, but in general this was in answer to what lawyers call a leading question. There was slight pain of the abdomen, increased on pressure, particularly in the region of the bladder, which organ suffered variously in its functions. In some the secretion of urine was suspended, in others it was free, but passed with pain and great effort. The action of the heart was feeble; the breathing unaffected; the face pale, expressive of much anxiety; the surface rather cold; the mental faculties unimpaired. Unluckily, the two fatal cases were not seen by any medical person; and we are, therefore, unable to state minutely the train of symptoms. We ascertained that the woman, in whose house were five sufferers, went away as in a gentle sleep, and that a few moments before death she had spoken and swallowed."

The woman died within three hours, and the other death was that of a watchman, who was found dead in his box six or seven hours after he had eaten the mussels. Post-mortem examination in these showed no abnormality. The stomach contained some of the food partially digested.

The explorer VANCOUVER reports four cases similar to those observed by COMBE. One of the sailors died in five and a half hours after eating the mussels.

In some recent cases reported by SCHMIDTMANN, as quoted by BRIEGER, the symptoms were as follows: Some dock hands and their families ate of cooked blue mussels which had been taken near a newly built dock. The symptoms appeared, according to the amount eaten, from soon after eating to several hours later. There was a sensation of constriction in the throat, mouth, and lips; the teeth were set on edge, as though sour apples had been eaten. There was dizziness, no headache; a sensation of flying, and an intoxication similar to that produced by alcohol. The pulse was hard, rapid (eighty to ninety), no elevation of temperature, the pupils dilated and reactionless. Speech was difficult, broken, and jerky. The limbs felt heavy; the hands grasped spasmodically at objects and missed their aim. The legs were no longer able to support the body, and the knees knocked together. There was nausea, vomiting, no abdominal pain, no diarrhœa. The hands became numb and the feet cold. The sensation of cold soon extended over the entire body, and in some the perspiration flowed freely. There was a feeling of suffocation, then a restful and dreamless sleep. One person died in one and three-quarters of an hour, another in three and one-half hours, and a third in five hours, after eating of the mussels.

In one of these fatal cases rigor mortis was marked and remained for twenty-four hours. The vessels of all the organs were distended, only the heart was empty. VIRCHOW concluded from the conditions observed that the blood had absorbed oxygen with great avidity. There was marked hyperæmia and swelling of the mucous membrane of the stomach and intestines, which VIRCHOW pronounced an enteritis. The spleen was enormously enlarged and the liver showed numerous hemorrhagic infarctions.

Many theories have been advanced to account for poisonous mussels. It was formerly believed that the effects were due to copper which the animals obtained from the bottoms of vessels; but, as CHRISTISON remarks, copper does not produce these symptoms. Moreover, CHRISTISON made analysis of the mussels which produced the symptoms observed by COMBE, and was unable to detect any copper. BOUCHARDAT found copper in some poisonous mussels, but he does not state the amount of the copper nor the source of the animals.

EDWARDS advanced the theory that the symptoms were wholly due to idiosyncrasy in the consumer. This may be true in some instances where only one or two of those partaking of the food are affected, but it certainly is not a tenable hypothesis in such instances as those reported by COMBE and SCHMIDTMANN, where a large number or all those who partook of the food were affected.

COLDSTREAM found the livers of the Leith mussels, as he thought, larger, darker, and more brittle than normal, and to this diseased condition he attributed the ill effects.

LAMOROUX, MOHRING, DE BEUME, CHENU, and DU RONDEAU have supposed that the poisonous effects were due to a particular species of medusæ upon which the mussels feed. DE BEUME found in the vomited matter of one person, suffering from mussel poisoning, some medusæ, and he states that these are most abundant during the summer, when mussels are most frequently found to be poisonous.

The theory of BURROW that the animal is always poisonous during the period of reproduction has been received with considerable credit. However, cases of poisoning have occurred at different seasons of the year.

CRUMPE, in 1872, suggested that there is a species of mussel which is in and of itself poisonous, and this species is often mixed with the edible variety. SCHMIDTMANN and VIRCHOW support this idea. They state that the poisonous species has a brighter shell, a sweeter, more penetrating, bouillon-like odor than the edible kind, also that the flesh of the former is yellow and that the water in which they are cooked is bluish. LOHMEYER also champions this opinion. This theory, however, is opposed by the majority of zoölogists. MÖBIUS states that the peculiarities of the supposed poisonous variety pointed out by VIRCHOW and SCHMIDTMANN are really due to the conditions under which the animal lives, the amount of salt in the water, the temperature of the water, whether it is moving or still water, the nature of the bottom, etc. Finally, MÖBIUS states that the sexual glands, which form the greater part of the mantle, are white in the male and yellow in the female. However, it has been shown later by SCHMIDTMANN and VIRCHOW that edible mussels may become poisonous if left in filthy water for fourteen days or longer, and, on the other hand, poisonous ones may become fit for food if kept for four weeks in good water.

Cats and dogs which have eaten voluntarily of poisonous mussels have suffered from symptoms similar to those observed in man; and rabbits have been poisoned by the administration of the water in which the food has been cooked. A rabbit which was treated in this manner by SCHMIDTMANN died within one minute. From these mussels BRIEGER extracted the ptomaïne mytilotoxine, which will be discussed in a subsequent chapter. This poison has a curare-like action. Whether or not those mussels which produce other symptoms also contain ptomaïnes, remains for future investigations to determine.

In 1887 three other cases of mussel poisoning, one fatal case, occurred at Wilhelmshaven, the place which supplied BRIEGER with the mussels from which he obtained mytilotoxine. SCHMIDTMANN has found that non-poisonous mussels placed in the waters of this bay soon become poisonous, and that the poisonous mussels from the bay placed in the open sea soon lose their poisonous properties. LINDER has found in the water of the bay and in the mussels living in it a great variety of protozoa, amœba, bacteria, and other lower organisms, which are not found in the water of the open sea nor in the non-poisonous mussel. He has also found that, if the water of the bay be filtered, non-poisonous mussels in it do not become poisonous. He therefore concludes that poisonous mussels are those which are suffering from disease due to residence in filthy water.

Brieger has tested dead and decomposed mussels taken from the open sea for mytilotoxine, with negative results.

Poisonous Oysters and Eels.—**Pasquier** reported cases of poisoning at Havre from the eating of oysters taken from an artificial bed which had been established near the outlet of a drain from a public water-closet. **Christison** says that an "unusual prevalence of colic, diarrhœa, and cholera" at Dunkirk was believed to have been traced to an importation of unwholesome oysters from the Normandy coast. **Vaughan** and **Novy** obtained tests for tyrotoxicon in the liquor of some decomposed oysters which had caused illness in many people at a church festival.

Virey states that many persons were attacked with violent pain and diarrhœa a few hours after eating a paté made of eels from a stagnant cattle-ditch near Orleans, also that similar cases have occurred in various parts of France, and that domestic animals have been killed by eating the remains of the poisonous dish.

Poisonous Fish.—While many species of fish are popularly regarded as poisonous, but little scientific work has been done in this line, and we are not prepared to say to what extent this popular idea is correct. **Miura** and **Takesaki** find that the ripe ovaries of *tetrodon rubripes* contain a substance which induces in rabbits acceleration of the respiratory movements, paralysis of the skeletal muscles, mydriasis, increased peristalsis of the intestines, and arrest of the heart.

The disease known as "kakke," which prevails from May to October in Tokio is, according to **Miura** and others, an intoxication due to the eating of fish, which belong to the *scombridæ*. The affection is generally chronic or subacute, seldom acute. The most characteristic symptom is paralysis of the diaphragm with consequent dyspnœa and disturbance of the action of the heart. Electrical stimulation of the diaphragm has proven to be the most successful treatment.

SAUSAGE POISONING.—This is also known as botulismus and allantiasis. While considerable diversity has been observed in symptoms of sausage poisoning, we cannot divide the cases into classes from their symptomatology as has been done in mussel poisoning. The first effects may manifest themselves at any time from one hour to twenty-four hours after eating of the sausage, and cases are recorded in which it is stated, no symptoms appeared until several days had passed. However, we must remember that trichinosis was frequently, in former times, classed as sausage poisoning, and it is highly probable that these cases of long delay in the appearance of the symptoms were really not due to putrefaction, but to the presence of parasites in the meat. A large majority of the one hundred and twenty-four cases more recently reported by MÜLLER sickened within twenty-four hours, and out of the forty-eight of these which were fatal, six died within the first twenty-four hours. At first there is dryness of the mouth, constriction of the throat, uneasiness in the stomach, nausea, vomiting, vertigo, indistinctness of vision, dilatation of the pupils, difficulty in swallowing, and usually diarrhœa, though obstinate constipation may exist from the first. There is, as a rule, a sensation of suffocation, and the breathing becomes labored. The pulse is small, thready, and rapid. In some cases the radial pulse may be imperceptible. Marked nervous prostration and muscular debility follow. These symptoms vary greatly in prominence in individual cases. The rechting and vomiting, which may be most distressing and persistent in some instances, in others are trivial at the beginning and soon cease altogether. The same is true of the diarrhœa. As a rule, the functions of the brain proceed normally, but there may be delirium, then coma and death. In some there are marked convulsive movements, especially of the limbs, in others paralysis may be an early and marked symptom. The pupils may dilate, then become normal and again dilate. There is frequently ptosis, and paralysis of the muscles of accommodation is not rare. Complete blindness has followed in a few instances.

The fatality varies greatly in different outbreaks. In 1820 KERNER collected reports of seventy-six cases, of which thirty-seven were fatal. In his next publication (1822) he increased the number to one hundred and fifty-five cases, with eighty-four fatal results. This gave a mortality of over fifty per cent., while in one outbreak reported by MÜLLER the mortality was less than two per cent.

A large proportion of the cases of sausage poisoning have occurred in Würtemberg and the immediately adjacent portions of Baden. This fact has, without doubt, been correctly ascribed to the methods there practised of preparing and curing the sausage. It is said to be common for the people to use the blood of the sheep, ox, and goat in the preparation of this article of diet. Moreover, the blood is kept sometimes for days in wooden boxes and at a high temperature before it is used. In these cases it is altogether likely that putrefaction progresses to the poisonous stage before the process of curing is begun. However, cases of poisoning have occurred from beef and pork sausages as well.

Moreover, the method of curing employed in Würtemberg favors putrefaction. A kind of sausage known as "blunzen" is made by filling the stomachs of hogs with the meat. In curing, the interior of this great mass is not acted upon, and putrefaction sets in. The curing is usually done by hanging the sausage in the chimney. At night the fire often goes out and the meat freezes. The alternate freezing and thawing render decomposition more easy. The interior of the sausage is generally the most poisonous. Indeed, in many instances those who have eaten of the outer portion have been unharmed, while those who have eaten of the interior of the same sausage have been most seriously affected.

Many German writers state that when a poisonous sausage is cut, the putrid portion has a dirty, grayish-green color, and a soft, smeary consistency. A disagreeable odor, resembling that of putrid cheese, is perceptible. The taste is unpleasant, and sometimes a smarting of the mouth and throat is produced. Post-mortem examination after sausage poisoning shows no characteristic lesion. It is generally stated that putrefaction sets in very tardily, but MÜLLER shows that no reliance can be placed upon this point, and states that out of forty-eight recorded autopsies, it was especially stated in eleven that putrefaction rapidly developed. In some instances there has been noticed hyperæmia of the stomach and intestinal canal, but this is by no means constant. The liver and brain have been reported as congested, but this would result from the failure of the heart, and would, by no means, be characteristic of poisoning with sausage.

VON FABER, in 1821, observed sixteen persons who were made sick by eating fresh, unsmoked sausage made from the flesh of a pig which had suffered from an abscess on the neck. Five of the patients died. The symptoms were as follows: There was constriction of the throat, difficulty in swallowing, retching, vomiting, colic-like pains, vertigo, hoarseness, dimness of vision, and headache. Later and in severer cases, there was complete exhaustion, and, finally, paralysis. The eyeballs were retracted, the pupils were sometimes dilated, then contracted; they did not respond to light; there was paralysis of the upper lids. The tonsils were swollen, but not as in tonsillitis. Liquids which were not irritating could be carried as far as the œsophagus, when they were then ejected from the mouth and nose with coughing. Solid foods could not be swallowed. On the back of the tongue and in the pharynx there was observed a puriform exudate.

Obstinate constipation existed in all, while the sphincter ani was paralyzed. The breathing was easy, but all had a croupous cough. The skin was dry. There was incontinence of urine. There was no delirium and the mind remained clear to the last.

Post-mortem examinations were held on four. The skin was rough—"goose-skin." The abdomen was retracted. The large vessels in the upper part of the stomach were filled with black blood. The contents of the stomach consisted of a reddish-brown, semi-fluid substance, which gave off a repugnant, acid odor. In one case the omentum was found greatly congested. The large intestine was very pale, and the right ventricle of the heart was filled with dark fluid blood.

SCHUZ cites thirteen cases of poisoning from liver sausage in which the symptoms differed from the foregoing in the following respects:

(1) In only one out of the thirteen was there constipation; all the others had numerous watery, typhoid-like stools.

(2) Symptoms involving the sense of sight were present in only three; in all the pupils were unchanged.

(3) The croupous cough was wholly wanting; though in many there was complete loss of voice. Difficulty of swallowing was complained of by only one.

(4) Delirium was marked in all; and in one the disturbance of the mental faculties was prominent for several weeks.

(5) There were no deaths.

(6) The time between eating the sausage and the appearance of the symptoms varied from eighteen to twenty-four hours, and the duration of sickness from one to four weeks; though in one case complete recovery did not occur until after two and one-half months.

The sausages were not smoked, and all observed a garlic odor, though no garlic had been added to the meat.

TRIPE reports sixty-four cases. The symptoms came on from three and one-half to thirty-six hours after eating. The stools were frequent, watery, and of offensive odor. In some there was delirium. One died. In the fatal case the hands and face were cold and swollen. The pulse was rapid and weak. The pupils were contracted, but responded to light. The small intestine was found inflamed.

HEDINGER reports the case of a man and a woman with the usual symptoms, but during recovery the dilatation of the pupils was followed by contraction. Birds ate of this sausage, and were not affected.

RÖSER reports cases in which there were found, after death, abscesses of the tonsils, a dark, bluish appearance of the mucous membrane of the pharynx, larynx, and bronchial tubes, dark redness of the fundus of the stomach, and circumscribed, gray, red, and black spots on the mucous membrane of the intestine. The liver was brittle and the spleen enlarged.

Many theories concerning the nature of the active principle of poisonous sausage have been advanced. It was once believed to consist of pyroligneous acid, which was supposed to be absorbed by the meat from the smoke used in curing it; but it was soon found that unsmoked sausage might be poisonous also. EMMERT believed that the active agent was hydrocyanic acid, and JÄGER'S theory supposed the presence of picric acid. But these acids are not found in poisonous sausage, and, moreover, their toxicological effects are wholly unlike those observed in sausage poisoning. As we have elsewhere seen, KERNER believed that he had found the poisonous principle in a fatty acid. This theory was supported by DANN, BÜCHNER, and SCHUMANN. KERNER believed the poison to consist of either caseic or sebacic acid, or both, while BÜCHNER named it acidum botulinicum; but the acids of the former proved to be inert, and that of the latter to have no existence. SCHLOSSBERGER first suggested that the poisonous substance is most probably basic in character, and he found an odoriferous, ammoniacal base which could not be found in good sausage, and which did not correspond to any known amides, imides, or nitril bases. However, this substance has not been obtained by anyone else, nor has it been demonstrated to be poisonous.

LIEBIG, DUFLAS, HIRSCH, and SIMON believed in the presence of a poisonous ferment. VAN DEN CORPUT described sarcina botulina, which was believed to constitute the active agent. MÜLLER, HOPPE-SEYLER, and others have found various microörganisms, and VIRCHOW, EICHENBERG, and others have examined microscopically the blood of persons poisoned with sausage. Recently, EHRENBERG has attempted to isolate the poisonous substance by employing BRIEGER'S method, but he obtained only inert substances.

Gaffky and Paak have made a thorough study of some sausage which poisoned a large number of people, among whom one, a strong man, died. The sausage was made of horse-flesh and liver. In the majority of the persons the symptoms came on within six hours and in one instance within half an hour. Many had a severe chill; some did not. The most prominent symptoms were headache, loss of appetite, pain in the bowels, vomiting and purging. In the fatal case, however, there was no vomiting. From the sausage Gaffky and Paak isolated a short bacillus, which when given by the mouth, subcutaneously or intravenously produced the above symptoms, with a fatal termination in most instances, in rabbits, guinea-pigs, mice, and apes. Gaffky and Paak were unable to isolate the chemical poison.

Poisonous Ham.—Under this head we shall not discuss cases of poisoning from trichina or other parasites, but shall refer only to those instances in which the toxic agent has originated in putrefactive changes. A number of such cases have been observed within the past ten years, but only a few of them have been investigated scientifically. The best known of these, as well as the most thoroughly studied, is the Wellbeck poisoning, which Ballard investigated successfully. In June, 1880, a large number of persons attended a sale of timber and machinery on the estate of the Duke of Portland at Wellbeck. The sale continued four days, and lunches were served by the proprietress of a neighboring hotel. The refreshments consisted of cold boiled ham, cold, boiled, or roasted beef, cold beefsteak pie, mustard and salt, bread and cheese, pickles and Chutney sauce. The drinks were bottle and draught beer, spirits, ginger beer, lemonade, and water. Many were poisoned, and Ballard obtained the particulars of seventy-two cases, among which there were four deaths. The symptoms are given by Ballard as follows:

"I propose to speak of the attacks under the name of 'diarrhœal illness,' because diarrhœa was the most constant of all the symptoms observed, and the other symptoms were in some respects so peculiar that I am indisposed to give to the disease any name otherwise generally recognized. As might have been anticipated from our experience of diseases in general, there were varieties in severity among the cases investigated; and symptoms strongly marked in some, were slightly marked or altogether wanting in others. Perhaps I shall do the best service by giving first a general sketch of the course of the illness, subsequently illustrating it by a description of a few well-marked cases.

"A period of incubation preceded the illness. In fifty-one cases where this could be accurately determined, it was twelve hours or less in five cases; between twelve and thirty-six hours in thirty-four cases; between thirty-six and forty-eight hours in eight cases; and later than this in only four cases. In many cases the first definite symptoms occurred suddenly, and evidently unexpectedly, but in some cases there were observed during the incubation more or less feeling of languor and ill health, loss of appetite, nausea, or fugitive, griping pains in the belly. In about a third of the cases the first definite symptom was a sense of chilliness, usually with rigors, of trembling, in one case accompanied by dyspnœa; in a few cases it was giddiness with faintness, sometimes accompanied by a cold sweat and tottering; in others, the first symptom was headache or pain somewhere in the trunk of the body, *e. g.*, in the chest, back, between the shoulders, or in the abdomen, to which part the pain, wherever it might have commenced, subsequently extended. In one case the first symptom noticed was a difficulty in swallowing. In two cases it was intense thirst. But however the attack may have commenced, it was usually not long before pain in the abdomen, diarrhœa, and vomiting came on, diarrhœa being of more certain occurrence than vomiting. The pain in several cases commenced in the chest or between the shoulders, and extended first to the upper and then to the lower part of the abdomen. It was usually very severe indeed, quickly producing prostration or faintness, with cold sweats. It was variously described as crampy, burning, tearing, etc. The diarrhœal discharges were in some cases quite unrestrainable, and (where a description of them could be obtained) were said to have been exceedingly offensive and usually of a dark color. Muscular weakness was an early and very remarkable symptom in nearly all the cases, and in many it was so great that the patient could only stand by holding on to something. Headache, sometimes severe, was a common and early symptom ; and in most cases there was thirst, often intense and most distressing. The tongue, when observed, was described usually as thickly coated with a brown, velvety fur, but red at the tip and edges. In the early stage the skin was often cold to the touch, but afterward fever set in, the temperature rising in some cases to 101°, 103°, and 104° F. In a few severe cases where the skin was actually cold, the patient complained of heat, insisted on throwing off the bedclothes, and was very restless. The pulse in the height of the illness became quick, counting in some cases 100 to 128. The above were the symptoms most frequently noted. Other symptoms occurred, however, some in a few cases, and some only in solitary cases. These I now proceed to enumerate. Excessive sweating, cramps in the legs, or in both legs and arms, convulsive flexion of the hands or fingers, muscular twitchings of the face, shoulders, or hands, aching pain in the shoulders, joints, or extremities, a sense of stiffness of the joints, prickling or tingling or numbness of the hands lasting far into convalescence in some cases, a sense of general compression of the skin, drowsiness, hallucinations, imperfection of vision, and intolerance of light. In three cases (one, that of a medical man) there was observed yellowness of the skin, either general or confined to the face and eyes. In one case, at a late stage of the illness, there was some pulmonary congestion, and an attack of what was regarded as gout. In the fatal cases, death was preceded by collapse like that of cholera, coldness of the surface, pinched, features and blueness of the fingers and toes and around the sunken eyes. The debility of convalescence was in nearly all cases protracted to several weeks.

"The mildest cases were characterized usually by little remarkable beyond the following symptoms, viz., abdominal pains, vomiting, diarrhœa, thirst, headache, and muscular weakness; any one or two of which might be absent."

The cause of this illness was traced conclusively to the hams eaten. KLEIN found in the meat a bacillus, cultures of which were used for inoculating animals. These inoculations were found generally to be followed by pneumonia. No attempt was made to isolate a ptomaïne.

Later, BALLARD reported fifteen cases with symptoms similar to the above, and with one death, from eating baked pork. Not all of those who ate of this pork were made sick. This might have been due to inequality in the putrefactive changes in different portions of the meat, or it may have been due to differences in temperature in various portions of the meat during the cooking. In the blood, pericardial fluid, and lungs of the fatal case, KLEIN observed bacilli similar to those discovered in the Wellbeck inquiry. Pneumonia was produced by inoculating guinea-pigs and mice with these bacilli.

In meat which poisoned a large number of persons, GÄRTNER found his bacillus enteritidis. The meat was from a cow which had a severe diarrhœa for two days before she was killed. Of twelve persons who ate the flesh raw, all were sick; while of those who ate of the cooked food a large per cent. were also affected. In the meat and in the spleen of a person who died from the effects of the poison, GÄRTNER found the bacillus, which proved fatal to animals. Good beef, inoculated with this bacillus and cooked some hours later, killed rabbits, guinea-pigs, and mice. The skin of the people who were poisoned and recovered peeled off. The period of incubation varied from two to thirty hours.

August 29, 1887, 256 soldiers and 36 citizens at Middleburg, Holland, were taken sick after eating meat from a cow which had been killed while suffering from puerperal fever. The symptoms were nausea, vomiting, purging, elevation of temperature, and prostration. In some there were observed dizziness, sleepiness, and dilatation of the pupil. After a few days these symptoms gradually disappeared, and in many an eczematous eruption of the lips gave annoyance. Pigs, cats, and dogs which ate of the offal of this animal were also made sick. Thorough cooking did not destroy the poison, and those who took soup and boullion made from the meat were affected like those who ate of the muscular fibre. In most of the cases the symptoms came on within twelve hours after eating the meat.

On a fête-day at Zurich, in 1839, 600 persons who were fed upon cold veal and ham were taken ill, with shivering, giddiness, vomiting, and diarrhœa. Some were delirious and others were salivated, the saliva being extremely fetid. In the worst cases there were involuntary stools, collapse, and death. The cause was traced to putrefactive changes in the meat.

SIEDLER reports an instance of four persons having been made sick by eating decomposed goose-grease. There were giddiness, prostration, and violent vomiting. No metallic poison could be found. The grease was rancid, of repulsive odor, and three ounces of it given to a dog produced the same symptoms which had been observed in the persons.

CHRISTISON reports a number of cases in which persons were seriously, a few fatally, affected by eating various kinds of meat which had undergone partial putrefaction.

OLLIVIER found six persons poisoned, four of them fatally, by eating of decomposed mutton. He also mentions the poisoning of a family of three with ham pie. Chemical analysis failed to reveal the presence of any poison.

BOUTIGNY, having failed to find any poison in the meat furnished at a festival, and to which the serious illness of many was attributed, made a meal of stuffed turkey furnished by the same dealer, but after a short time his countenance became livid, his pulse small and feeble, a cold sweat bathed his body, and violent vomiting and purging followed. His recovery was slow.

GEISELER observed nausea, vomiting, purging, and delirium after eating of bacon which was imperfectly cured.

POISONOUS CANNED MEATS.—Cases of poisoning from eating canned meats have become quite frequent. Although it may be possible that in some instances the untoward effects result from metallic poisoning, in the great majority of cases the poisonous principles are formed by putrefactive changes. In many instances it is probable that decomposition begins after the can is opened by the consumer. In others, the canning is carelessly done and putrefaction is far advanced before the food reaches the consumer. In still other instances, the meat may be taken from diseased animals, or it may undergo putrefactive changes before the canning. What is true of canned meats is also true of canned fruits and vegetables.

Dr. ASHWORTH, of Smithland, Iowa, has reported to us three fatal cases of poisoning from canned apricots. An infant, which was only eight days old, and which must have received the poison from its mother's breasts, died within a few hours. The mother died forty-three hours after eating the apricots, and the father on the sixth day. The symptoms corresponded with those of poisoning by tyrotoxicon. However, it seems that no analysis was made, and these may have been cases of mineral poisoning.

POISONOUS CHEESE.—In 1827 HÜNNEFELD made some analyses of poisonous cheese, and experimented with extracts upon the lower animals. He accepted the ideas of KERNER in regard to poisonous sausage in a somewhat modified form, and thought the active agents to be sebacic and caseic acids. About the same time, SERTÜRNER, making analyses of poisonous cheese for WESTRUMB, also traced the poisonous principles, as he supposed, to these fatty acids. We see from this that during the first part of the present century the fatty acid theory, as it may be called, was generally accepted.

In 1848, CHRISTISON, after referring to the work of HÜNNEFELD and SERTÜRNER, made the following statement: "His (Hünnefeld's) experiments, however, are not quite conclusive of the fact that these fatty acids are really the poisonous principles, as he has not extended his experimental researches to the caseic and sebacic acids prepared in the ordinary way. His views will probably be altered and simplified if future experiments should confirm the late inquiries of BRACONNOT, who has stated that PROUST'S caseic acid is a modification of acetic acid combined with an acrid oil."

In 1852 SCHLOSSBERGER made experiments with the pure fatty acids and demonstrated their freedom from poisonous properties. These experiments have been verified repeatedly, so that now it is well known that all the fatty acids obtainable from cheese are devoid of poisonous properties.

It may be remarked here, that there is every probability that the poisonous substance was present in the extracts obtained by the older chemists. Indeed, we may say that this is a certainty, since the administration of these extracts to cats was, in some instances at least, followed by fatal result. The great mass of these extracts consisted of fatty acids, and as the chemists could find nothing else present, they very naturally concluded that the fatty acids themselves constituted the poisonous substance.

Since the overthrow of the fatty acid theory, various conjectures have been made, but none worthy of consideration.

We make the following quotations from some of the best authorities who wrote during the first half of the past decade upon this subject:

HILLER says: "Nothing definite is known of the nature of cheese poison. Its solubility seems established from an observation by HUSEMANN, a case in which the poison was transmitted from a nursing mother to her child."

HUSEMANN wrote as follows: "The older investigations of the chemical nature of cheese poison, which led to the belief of putrefactive cheese acids and other problematic substances, are void of all trustworthiness, and the discovery of the active principle of poisonous cheese may not be looked for in the near future, on account of the proper animals for controlling the experiments with the extracts, as dogs can eat large quantities of poisonous cheese without its producing any effect."

BRIEGER stated in 1885: "All kinds of conjectures concerning the nature of this poison have been formed, but all are even devoid of historical interest; because they are not based upon experimental investigations. My own experiments toward solving this question have not progressed very far."

In the above quotation we think that BRIEGER has hardly done justice to the work of HÜNNEFELD and SERTÜRNER. Their labors can hardly be said to be wholly devoid of historical interest, and they certainly did employ the experimental method of inquiry.

In the years 1883 and 1884 there were reported to the Michigan State Board of Health about three hundred cases of cheese poisoning. As a rule, the first symptoms appeared within from two to four hours after eating the cheese. In a few the symptoms were delayed from eight to ten hours and were very slight. The attending physicians reported that the gravity of the symptoms varied with the amount of cheese eaten, but no one who ate of the poisonous cheese wholly escaped. One physician reported the following symptoms: "Everyone who ate of the cheese was taken with vomiting, at first of a thin, watery, later a more consistent reddish-colored substance. At the same time the patient suffered from diarrhœa with watery stools. Some complained of pain in the region of the stomach. At first the tongue was white, but later it became red and dry, the pulse was feeble and irregular; countenance pale, with marked cyanosis. One small boy, whose condition seemed very critical, was covered all over the body with bluish spots."

Dryness and constriction of the throat were complained of by all. In a few cases the vomiting and diarrhœa were followed by marked nervous prostration, and in some dilatation of the pupils was observed.

Notwithstanding the severity of the symptoms in many, there was no fatal termination among these cases, though several deaths from cheese poisoning in other outbreaks have occurred. Many of the physicians at first diagnosed the cases from the symptoms as due to arsenical poisoning, and on this supposition some administered ferric hydrate. Others gave alcohol and other stimulants and treated upon the expectant plan.

VAUGHAN, to whom the cheese was sent for analysis, made the following report: "All of these three hundred cases were caused by eating of twelve different cheeses. Of these, nine were made at one factory, and one each at three other factories. Of each of the twelve I received smaller or larger pieces. Of each of ten I received only small amounts. Of each of the other two I received about eighteen kilogrammes. The cheese was in good condition and there was nothing in the taste or odor to excite suspicion. However, from a freshly cut surface there exuded numerous drops of a slightly opalescent fluid which reddened litmus paper instantly and intensely. Although, as I have stated, I could discern nothing peculiar in the odor, if two samples, one of good, the other of poisonous cheese, were placed before a dog or cat, the animal would invariably select the good cheese. But if only poisonous cheese was offered, and the animal was hungry, it would partake freely. A cat was kept seven days and furnished only poisonous cheese and water. It ate freely of the cheese and manifested no untoward symptoms. After the seven days the animal was etherized and abdominal section was made. Nothing abnormal could be found. I predicted, however, in one of my first articles on poisonous cheese, that the isolated poison would affect the lower animals. As to the truth of this prediction we will see later.

"My friend, Dr. STERNBERG, the eminent bacteriologist, found in the opalescent drops above referred to numerous micrococci. But inoculations of rabbits with these failed to produce any results.

"At first I made an alcoholic extract of the cheese. After the alcohol was evaporated in vacuo at a low temperature a residue consisting mainly of fatty acids remained. I ate a small bit of this residue, and found that it produced dryness of the throat, nausea, vomiting, and diarrhœa. The mass of this extract consisted of fats and fatty acids, and for some weeks I endeavored to extract the poison from these fats, but all attempts were unsuccessful. I then made an aqueous extract of the cheese, filtered this, and drinking some of it, found that it also was poisonous. But after evaporating the aqueous extract to dryness on the water-bath at 100°, the residue thus obtained was not poisonous. From this I ascertained that the poison was decomposed or volatilized at or below the boiling-point of water. I then tried distillation at a low temperature, but by this the poison seemed to be decomposed.

"Finally, I made the clear, filtered aqueous extract, which was highly acid, alkaline with sodium hydrate, agitated this with ether, removed the ether, and allowed it to evaporate spontaneously. The residue was highly poisonous. By re-solution in water and extraction with ether, the poison was separated from foreign substances. As the ether took up some water, this residue consisted of an aqueous solution of the poison. After this was allowed to stand for some hours in vacuo over sulphuric acid, the poison separated in needle-shaped crystals. From some samples the poisoned crystallized from the first evaporation of the ether, and without standing in vacuo. This happened only when the cheese contained a comparatively large amount of the poison. Ordinarily, the microscope was necessary to detect the crystalline shape. From sixteen kilogrammes of one cheese, I obtained about 0.5 gramme of the poison, and in this case the individual crystals were plainly visible to the unaided eye. From the same amount of another cheese I obtained only about 0.1 gramme, and the crystals in this case were not so large. I have no idea, however, that by the method used all the poison was separated from the cheese."

To this ptomaïne VAUGHAN has given the name tyrotoxicon (*τυρος*, cheese, and *τοξικον*, poison). Its chemistry will be discussed in a subsequent chapter.

During 1887, WALLACE found tyrotoxicon in two samples of cheese which had caused serious illness. The first of these came from Jeanesville, Pa., and the symptoms as reported to WALLACE by DOOLITTLE, who had charge of the cases, were as follows: "There were at least fifty persons poisoned by this cheese. There were also eight others who ate of the cheese, but felt no unpleasant effects; whether this was due to personal idiosyncrasy, or to an uneven distribution of the poison throughout the cheese, I am unable to say.

"The majority, however, comprising fifty or sixty persons, were seized, in from two to four hours after eating the cheese, with vertigo, nausea, vomiting, and severe rigors, though varying in their order of appearance and in severity in different cases. The vomiting and chills were the most constant and severe symptoms in all the cases, and were soon followed by severe pain in the epigastric region, cramps in the feet and lower limbs, purging and griping pain in the bowels, a sensation of numbness or pins and needles, especially in the limbs, and lastly, very marked prostration, amounting almost to collapse in a few cases.

"The vomit at first consisted of the contents of the stomach, and had a strong odor of cheese; afterward it consisted of mucus, bile, and in three or four of the severer cases blood was mixed with the mucus in small quantities. Microscopic examination of the same was not made, but to the eye it appeared as such. The vomiting and diarrhœa lasted from two to twelve hours; the rigors and muscular cramps, one to two hours. The diarrhœal discharges, at first fecal, became later watery and light colored. No deaths occurred, and for the most part the effects were transient, and all that remained on the following day were the prostration and numbness; the latter occurred in about one-half the cases, and disappeared in from one to three days.

"Children, as a rule, seemed to suffer less than adults, and, of course, it was not possible to elicit as definite symptoms from them. The suddenness of the attack was remarked by all, some feeling perfectly well until the moment of attack. Nor did the symptoms seem to be in proportion to the amount of cheese taken; some of the severest cases declared they had not eaten more than a cubic inch of it. One of the severest cases was about six and one-half months pregnant, but no interference with pregnancy occurred. All the cheese which caused the sickness came from the same piece."

The second sample of cheese examined by WALLACE came from Riverton, N. J. This outbreak included a smaller number of persons, all of whom recovered.

WOLFF has detected tyrotoxicon in cheese which poisoned several persons at Shamokin, Pa. The pores of this cheese were found filled with a grayish-green fungoid growth, though it is not supposed that this fungus was connected in any way with the poisonous nature of the cheese. Tests were made for mineral poison with negative results, after which tyrotoxicon was recognized both by chemical and physiological tests. "A few drops of the liquid (extract), placed on the tongue of a young kitten, produced prompt emesis and numerous watery dejections with evident depression and malaise of the animal. A larger cat was similarly affected by it, though the depression and malaise were not so marked nor so long continued."

Cheese poisoning caused the death of several children in the neighborhood of Heiligenstadt, in 1879, and there were many fatal cases from the same cause in Pyrmont, in 1878. Unfortunately we have not been able to find any detailed account of either the symptoms or the post-mortem appearances in these cases.

EHRHART has published the history of some cases of poisoning from cheese, of which the following is an abstract: The family of a workman, consisting of eight persons, ate for supper 600 grammes (about eighteen ounces) of Limburger cheese. The rind was covered with a heavy mould, while the interior had become fluid from putrefaction, and was of bitter taste. Three ate only of the mouldy rind, and these remained well. The next morning, the five who had eaten of the inner portion suffered from vertigo, nausea, vomiting, and abdominal pains; no stool. The father had convulsive movements of all the extremities. The pupils were dilated, and did not respond to light; there were double vision, cold sweat, skin cyanotic, abdomen distended, difficulty in swallowing, delirium, mild trismus, and temperature 40° C. (104° F.). The temperature of the mother, on account of the great collapse, was subnormal. She had no convulsive movements, but there was prolonged loss of consciousness. The pulse was small and thready, and threatened paralysis of the heart. Recovery was very slow. The others suffered only from gastro-enteric symptoms. EHRHARDT discusses the question as to whether these symptoms were due to tyrotoxicon, or to infection with microörganisms; but as we have not had access to his original paper, we do not know what his conclusions are. However, there cannot be much doubt that in those cases in which the organism is taken into the alimentary canal, it continues the elaboration of its poisonous products.

In 1890 VAUGHAN made the following additional report on poisonous cheese:

"During the past two or three years we have received at the Hygienic Laboratory of Michigan University a number of samples of cheese which, it was claimed, had caused nausea and vomiting in those eating of them, and in which we were unable to detect tyrotoxicon. Some of these samples produced vomiting and purging in cats and dogs to which the cheese was fed directly. The evidence that these samples had been the actual cause of the sickness among the people who had eaten of them was thus confirmed by the experiments upon the animals; but inasmuch as we were unable to detect the poison, we were compelled to report as follows:

"'The poisonous character of the cheese has been proven by experiments upon animals, but we have failed to demonstrate the nature of the poison. Tyrotoxicon could not be detected.'

"One sample of this class was found by NOVY to be very poisonous. Some of this cheese was covered with absolute alcohol, and after standing in a dish for some weeks the alcohol was allowed to evaporate, then 100 grammes of the cheese was fed to a young dog and caused its death within a few hours. Sterilized milk to which a small bit of the cheese was added, after standing in the incubator at 35°.

for twenty-four hours, became so poisonous that 100 c. c. of it introduced into the stomach of a full-grown cat caused death. NOVY made plate cultures from the cheese and from the spleen and liver of the dead animals, and succeeded in identifying one germ as common to both. Sterilized milk inoculated with a pure culture of this germ, and kept in the incubator, proved fatal to cats. But with the advent of cold weather the germ lost its toxicogenic properties, which were not restored by subsequent cultivation in the incubator.

" In a second class of samples, the poisonous character of the cheese was not confirmed by direct feeding. Cats, rats, and dogs were fed with the same quantities as above, without any appreciable effect. The report made upon the samples was as follows:

" ' Animals fed upon the cheese were not affected. Tyrotoxicon could not be found. The sickness in the people was probably due to some other cause.'

" The last sentence of this report was probably wrong, as will be shown from the following experiment. Two kilogrammes of a cheese of this class was extracted repeatedly with absolute alcohol. The part insoluble in alcohol was then extracted with water. The aqueous extract, after filtration, was allowed to fall slowly into three times its volume of absolute alcohol. A voluminous, flocculent precipitate resulted. After twenty-four hours the supernatant fluid was decanted, and the precipitate was dissolved in water and re-precipitated with absolute alcohol; then it was collected and speedily dried on porous plates. A small bit of this precipitate was dissolved in water; and forty drops of this solution, injected under the skin on the back of cats, produced invariably within one hour vomiting and purging. After the partial collapse which followed the vomiting and purging, and which was evidenced by the animal sitting with its chin resting on the floor, recovery gradually followed. The same amount of the solution injected into the abdominal cavity of white rats rendered the animals within ten or fifteen minutes perfectly limp, and the only evidence of life observed was rapid respiratory movements. The rats lay upon their sides, and could be handled without manifesting any attempt at movement. In this condition some died after three or four hours, while others, after lying in this position for from eighteen to twenty-four hours, gradually improved, and after some days seemed to be wholly recovered.

"This substance belongs to the so-called poisonous albumins. From its aqueous solutions it is not precipitated by heat or nitric acid, singly or combined. Its solutions respond to the biuret test. It is not precipitated by saturation with sodium sulphate, nor by a current of carbonic acid gas; therefore, it is not a globulin. It is precipitated by saturation with ammonium sulphate; and this fact removes it from the peptones.

"That animals were not affected when fed with the whole cheese may be explained by the supposition that they did not in this manner get enough of the poison to affect them. It cannot be said positively that the samples of cheese of the first class mentioned above owe their poisonous properties to this substance. We have not had the opportunity of testing samples of this class since the recognition of the poisonous proteid in those of the second class. Four samples of the latter have been tested for the poisonous albumin with positive results.

"It may be found that traces of this poison exist in all samples of green cheese. This point will be investigated.

"It is highly probable that the poisonous effects of some samples of sausage and meat are due to similar products of bacterial activity."

In reference to the poisonous proteids in cheese and other articles of food the following interesting questions arise: How is the poisoning explained? Is it not generally supposed that poisonous proteids are not absorbable from mucous membranes? MITCHELL and REICHERT showed that the venom of serpents may be absorbed from mucous membranes; especially did they find this to be true of the poisonous peptone of the cobra. It may be, however, that the bacteria, which are in the cheese and to which the formation of the poisonous proteids is due, find their way through the intestinal walls and form their poisonous products within the spleen and other organs. The fact that NOVY found the bacteria in the spleen and liver of the animals experimented upon confirms this view.

POISONOUS MILK.—In 1885 VAUGHAN found tyrotoxicon in milk which had stood in a well-stoppered bottle for about six months. It was presumed that this milk was, when first obtained, normal in composition, but since this was not known with certainty, the following experiments were made: Several gallon bottles were filled with normal milk, tightly closed with glass stoppers, and allowed to stand at the ordinary temperature of the room. From time to time a bottle was opened and the test for tyrotoxicon was made. These tests were followed by negative results until about three months after the experiment was begun. Then the poison was obtained from one of the bottles. The coagulated milk was filtered through paper. The filtrate, which was colorless and decidedly acid in reaction, was rendered feebly alkaline by the addition of potassium hydrate and agitated with ether. After separation, the ethereal layer was removed with a pipette, passed through a dry filter-paper in order to remove a flocculent, white substance which floated in it, and then allowed to evaporate spontaneously. If necessary, this residue was dissolved in water and again extracted with ether. As the ether takes up some water, there is usually enough of the latter left after the spontaneous evaporation of the ether to hold the poison in solution, and in order to obtain the crystals this aqueous solution must be allowed to stand for some hours in vacuo over sulphuric acid.

From one-half gallon of the milk there was obtained quite a concentrated aqueous solution of the poison after the spontaneous evaporation of the ether. Ten drops of this solution placed in the mouth of a small dog, three weeks old, caused within a few minutes frothing at the mouth, retching, the vomiting of frothy fluid, muscular spasms over the abdomen, and after some hours watery stools. The next day the dog seemed to have partially recovered, but was unable to retain any food. This condition continuing for two or three days the animal was killed with chloroform. No examination of the stomach was made.

In 1886 Newton and Wallace obtained tyrotoxicon from milk and studied the conditions under which it forms. Their report is of so much value that the greater part of it is herewith inserted.

"On August 7th twenty-four persons, at one of the hotels at Long Branch, were taken ill soon after supper. At another hotel, on the same evening, nineteen persons were seized with the same form of sickness. From one to four hours elapsed between the meal and the first symptoms. The symptoms noticed were those of gastro-intestinal irritation, similar to poisoning by any irritating material—that is, nausea, vomiting, cramps, and collapse; a few had diarrhœa. Dryness of the throat and burning sensation in the œsophagus were prominent symptoms.

"While the cause of the sickness was being sought for, and one week after the first series of cases, thirty persons at another hotel were taken ill with precisely the same symptoms as noticed in the first outbreak.

"When the news of the outbreak was published one of us immediately set to work, under the authority of the State Board of Health, to ascertain the cause of the illness. The course of the investigation was about as follows:

"The character of the illness indicated, of course, that some article of food was the cause, and the first part of our task was to single out the one substance that seemed at fault. The cooking utensils were also suspected, because unclean copper vessels have often caused irritant poisoning. Articles of food, such as lobsters, crabs, blue fish, and Spanish mackerel, all of which at times, and with some persons very susceptible to gastric irritation have produced toxic symptoms, were looked for, but it was found that none of these had been eaten at the time of the outbreak. The cooking vessels were examined, and all were found clean and bright, and no evidence of corrosion was presented.

"Further inquiry revealed the fact that all who had been taken ill had used milk in greater or less quantities, and that persons who had not partaken of milk escaped entirely; corroborative of this, it was ascertained that those who had used milk to the exclusion of all other food were violently ill. This was prominently noticed in the cases of infants fed from the bottle, when nothing but uncooked milk was used. In one case an adult drank about a quart of the milk, and was almost immediately seized with violent vomiting followed by diarrhœa, and this by collapse. Suffice it to say, that we were able to eliminate all other articles of food and to decide that the milk was the sole cause of the outbreak.

"Having been able to determine this, the next step was to discover why that article should, in these cases, cause so serious a form of sickness.

"The probable causes which we were to investigate were outlined as follows: (1) Some chemical substance, such as borax, boric acid, salicylic acid, sodium bicarbonate, sodium sulphate, added to preserve the milk or to correct acidity. (2) The use of polluted water as an adulterant. (3) Some poisonous material accidentally present in the milk. (4) The use of milk from diseased cattle. (5) Improper feeding of the cattle. (6) The improper care of the milk. (7) The development in the milk of some ferment or ptomaïne, such as tyrotoxicon.

"At the time of the first outbreak we were unable, unfortunately, to obtain any of the noxious milk, as that unconsumed had been destroyed; but at the second outbreak a liberal quantity was procured.

"It was soon ascertained that one dealer had supplied all the milk used at the three hotels where the cases of sickness had occurred. His name and address having been obtained, the next step in the investigation was to inspect all the farms, and the cattle thereon, from which the milk was taken. We also learned that two deliveries at the hotels were made daily, one in the morning and one in the evening; that the milk supplied at night was the sole cause of the sickness, and that the milk from but one of the farms was at fault. The cows on this farm were found to be in good health, and, besides being at pasture, were well fed with bran, middlings, and corn-meal.

"So far we had been able to eliminate as causes diseased cattle and improper feeding, and we were then compelled to consider the other possible sources of the toxic material.

"While the inspection of the farms was being made, the analysis of the milk was in progress. The results of this showed that no chemical substance had been added to the milk, that it was of average composition, that no polluted water had been used as a diluent, and that no poisonous metals were present. This result left us nothing to consider but two probable causes: improper care of the milk, and the presence of a ferment.

"As to the former, we soon learned much. The cows were milked at the unusual and abnormal hours of midnight and noon, and the noon's milking—that which alone was followed by illness—was placed, while hot, in the cans, and then, without any attempt at cooling, carted eight miles during the warmest part of the day in a very hot month.

"This practice seemed to us sufficient to make the milk unpalatable, if not injurious, for it is well known that when fresh milk is closed up in a tight vessel and then deposited in a warm place, a very disagreeable odor and taste are developed. Old dairymen speak of the animal heat as an entity, the removal of which is necessary in order that the milk shall keep well and have a pleasant taste. While we do not give this thing a name, we are fully convinced that milk should be thoroughly cured by proper chilling and aëration before it is transported any distance or sold for consumption in towns or cities.

"This opinion is based on a study of the methods prevalent among experienced dairymen, who ship large quantities of milk to our great cities. The usual practice is to allow the milk to stand in open vessels, surrounded by ice or cold water, for from eight to twelve hours before transportation, and when placed on the cars it has a temperature of from 50° to 60° F., and is delivered to consumers in a perfectly sweet condition. The city of New York receives about 200,000 gallons each day from the surrounding country, and much of it brought in by the railroads has been on the cars for a time varying from six to twelve hours, yet we seldom hear of any of this milk undergoing the peculiar form of fermentation set up in the Long Branch milk. We may account for this by assuming that the proper care of the milk after it was taken from the cow, and the low temperature at which it was kept, have prevented the formation of any ferment; this opinion seems to be endorsed by all dairymen and managers of large creameries with whom we have consulted. They all agree in stating that milk maintained at a low temperature can be kept sweet and in good condition for many days.

"We have dwelt on this branch of our topic somewhat extensively, because we are fully persuaded that the improper care of the milk had much to do with the illness it produced.

"The results of our inquiry having revealed so much, we next attempted to isolate some substance from the poisonous milk, in order that the proof might be more evident. A quantity of the milk that had caused sickness in the second outbreak was allowed to coagulate, was then thrown on a coarse filter, and the filtrate collected. This latter was highly acid, and was made slightly alkaline by the addition of potassium hydrate. This alkaline filtrate was now agitated with an equal volume of pure, dry ether, and allowed to stand for several hours, when the ethereal layer was drawn off by means of a pipette. Fresh ether was added to the residuum, then agitated, and, when separated, was drawn off and added to the first ethereal extract. This was now allowed to evaporate spontaneously, and the residue, which seemed to contain a small amount of fat, was treated with distilled water and filtered, the filtrate treated with ether, the ethereal solution drawn off and allowed to evaporate, when we obtained a mass of needle-shaped crystals. This crystalline substance gave a blue color with potassium ferricyanide and ferric chloride, and reduced iodic acid. The crystals, when placed on the tongue, gave a burning sensation. A portion of the crystals was mixed with milk and fed to a cat, when, in the course of half an hour, the animal was seized with retching and vomiting, and was soon in a condition of collapse, from which it recovered in a few hours.

"We are justified in assuming, after weighing well all the facts ascertained in the investigation, that the sickness at Long Branch was caused by poisonous milk, and that the toxic material was tyrotoxicon.

"The production of this substance was no doubt due to the improper management of the milk—that is, too long a time was allowed to elapse between the milking and the cooling of the milk, the latter not being attended to until the milk was delivered to the hotel; whereas, if the milk had been cooled immediately after it was drawn from the cows, fermentation would not have ensued, and the resulting material, tyrotoxicon, would not have been produced."

In the same year, SCHEARER found the same poison in the milk used by, and the vomited matter of, persons made sick at a hotel at Corning, Iowa.

In 1887, FIRTH, an English army surgeon stationed in India, reported an outbreak of milk poisoning among the soldiers of his garrison. From the milk he separated, by VAUGHAN'S method, tyrotoxicon. He also obtained tyrotoxicon from milk which had been kept for some months in stoppered bottles, as had been previously done by Vaughan. (See page 62.)

In 1887, MESIC and VAUGHAN observed four cases of milk poisoning, three of which terminated fatally, and NOVY and VAUGHAN obtained tyrotoxicon from the milk, and from the contents of the intestine in one of the fatal cases. VAUGHAN reports these cases as follows:

"September 23, 1887, I was visited by Dr. A. G. Mesic, of Milan, Michigan, who informed me that he had four members of a family under his charge, all of whom were seriously ill with peculiar symptoms which he believed to be caused by tyrotoxicon. Since Dr. Mesic has written out for me the history of these cases, I will insert his report in full, as follows:

"'Saturday, September 17, while passing the residence of S. H. Evans, a respectable farmer, I was called in to see him. I found him—a man of about fifty years, spare and muscular—vomiting severely, with flushed face, but with a temperature of 96° F. There was marked throbbing of the abdominal aorta; the tongue had a white, heavy coating, and the breathing was very labored. I set to work with the ordinary remedies to allay the vomiting, which had already continued for some hours. The vomited matters were colored with bile. Pupils were dilated, and a rash resembling that of scarlatina, but coarser, covered the chest, forearms, and legs below the knees, while the abdomen and thighs remained unaffected. As the bowels had not been moved since the beginning of the attack, I administered a purgative dose of calomel with a little podophyllin and rhubarb. On Sunday a small stool resulted. During that day and night, and the following day, the retching and vomiting continued. Small doses of carbolic acid seemed to give the most relief. After the movement of the bowels the symptoms were somewhat more promising; but a heavy and unfavorable stupor was observable and persistent.

"'On Sunday the coating of the tongue remained very thick, and had changed to a dark brown color. At first I thought that his symptoms indicated a depressed condition, which I had known in one instance to precede typhoid fever. However, after a few days, I concluded that I must look for the cause of the condition among the poisons; but I could think of no one poison which would be likely to produce all the symptoms observed. During Monday, Tuesday, and Wednesday, there was but little change, and the treatment was continued.

"'On Thursday morning I found the son Arthur, a lad of eighteen years, strong and vigorous, suffering with the same symptoms, only in a more violent form. After supper on Wednesday evening he was taken with nausea and vomiting. He had no rash, but the symptoms were otherwise identical with those of the father, except in being more severe. I gave a cathartic, which acted only slightly.

"'At my evening visit I found Mrs. Evans, a lady of about forty-five, previously in good health, with the same symptoms. In this case the stupor was more marked from the first. I was unable at any time to obtain any cathartic action in this case. Copious enemata of warm water were used, but succeeded only in washing some hardened lumps from the rectum. By this time I had concluded that the poison was most likely tyrotoxicon.

"'On Friday morning the only remaining member of the family at home, Miss Alma, sixteen years of age, was affected in the same way as the others. On that day I went to Ann Arbor, and gave a history of the cases so far to Dr. Vaughan, who, from the symptoms, thought that my diagnosis was most probably correct, and he advised with me as to treatment, which I carried out. I gave two grains of sodium salicylate every four hours, and used small doses of the tonics and stimulants, quinine, nux vomica, digitalis, whiskey, and the aromatic spirits of ammonia. On Saturday the symptoms in all remained unimproved, and in the mother and son the stupor and labored breathing grew more marked.

"'On Sunday, I again went to Ann Arbor, and brought Dr. Vaughan with me to see the patients. The temperature of the mother on Sunday was as low as 94° F., and that of the son 95° F. Dr. Vaughan agreed with me as to diagnosis and treatment. Sunday evening the patients were all removed to the house of a neighbor, about forty rods distant (the reasons for this will be given later). Dr. Vaughan and I both expressed the fear that the mother, and possibly the son, would not live through the night. Both of these rapidly grew worse, and the son died at 7.45 A.M. and the mother at 4 P.M., Monday.

"'During Monday the daughter rapidly grew worse, and at the time of her mother's death could not be aroused, and practically she remained unconscious from that time on. The father was very weak, but retained his consciousness all the time. Convulsive movements of the limbs had been noticed in the son, but not in the mother. These now became more marked in the daughter, who remained in the heavy stupor, with labored breathing, until 5 P.M. Thursday, when she died.

"'Mr. Evans has slowly improved, and now, October 18th, is able to walk about the room. The sodium salicylate, even in the small doses used, seemed to cause severe headache; so apparent was this that the drug was discontinued, and drop doses of amyl nitrite, given every hour, seemed to relieve the pain in the head. His temperature remained below the normal until Thursday, October 14th, when it reached the normal. After this it was found once as high as 99.5° F., then 99° F., then again normal, where it remains.

"'All complained of a burning constriction in the throat, and difficulty in swallowing, and all, as long as they were conscious, frequently called for ice. In all the pulse was rapid and feeble, and death seemed to result from failure of the heart. Those who died voided urine involuntarily, while Mr. Evans passed small quantities frequently, and for this buchu and uva ursa were given. During his convalescence small doses of morphine were given, as he was unable to sleep, and became very restless. He is now taking teaspoonful doses of the elixir of calisaya and iron every four hours.'

"As stated above by Dr. Mesic, I first saw these patients Sunday, September 25th. On a sofa in the room we found the daughter, Alma. She had been vomiting during the day, and seemed much exhausted. She was not inclined to talk, and seemed to be in a stupor, though when spoken to she responded rationally. Her pupils were slightly dilated, her tongue coated, her pulse 120 and weak, her face flushed, and a violent throbbing could be felt over the abdomen, which was retracted. Her temperature was 96° F.

"In another room were the father, mother, and son, two of them dying. The father was rational, and talked with some freedom when I asked as to the kind of food they had been eating, etc. His pupils were normal. His face could not be said to present any peculiar feature. His pulse was rapid, breathing somewhat labored, and the throbbing of the abdominal aorta was plainly felt. The abdomen was retracted, and there was no pain on pressure. He complained of a burning constriction of the throat, swallowed with difficulty, and said that his throat and stomach felt as though they were on fire.

"The mother lay perfectly still with eyelids closed, as if in a deep sleep. Her pulse was rapid, her face had a livid flush, her breathing was about 35 per minute, and labored. The skin was cool, but neither abnormally moist nor specially dry and harsh. She could not be aroused. In fact, she was comatose.

"The son rolled uneasily from one side of the bed to the other. His breathing, also, was very labored. His eyelids were closed, and the pupils were markedly dilated—did not respond to light. He could not be aroused. In mother and son, as well as in father and daughter, the abdomen was retracted, and the throbbing of the abdominal aorta was easily felt.

"Now, to what were these symptoms due? They were certainly those of some poison. Dr. Mesic had brought me some of the vomited matter, which I tested thoroughly for mineral poisons, with negative results. The symptoms certainly were not those of morphine, strychnine, digitalis, or aconite. They did have some resemblance to those of belladonna, but yet they were not the symptoms of belladonna. The pupils were not as widely dilated as they would be in belladonna poisoning. There was in none of these persons the active delirium of belladonna poisoning. There was no picking at the clothing, no grasping of imaginary objects in the air, no hallucinations of vision. Surely it could not be any vegetable alkaloid with which I was familiar.

"On the other hand, we know that nausea, vomiting, headache, dilatation of the pupil, rapid pulse, heavy breathing, constipation, and great prostration, with stupor, do occur in cases of poisoning with certain ptomaïnes. Therefore we began to look for conditions which would be favorable for the production of putrefactive alkaloids. These conditions we were not long in finding.

"The family, which consisted of the four persons sick, and of a daughter about twenty years of age, who was away from home at the time when the others were taken ill, and for some months before that time, was evidently a tidy one. This was shown by their personal appearance, and by the clothing and bedding. But the house in which they lived was very old, and very much decayed. Mr. Evans had purchased the farm six years ago; and for some three years past, at least, they had been troubled every now and then, one or more of the family, with nausea and vomiting, followed by more or less prostration. But in no instance, up to the present illness, had the symptoms been sufficient to cause them to summon a physician. The family had worked hard in order to pay for the farm, and had determined to make the old house do until they were out of debt. Even before this family had moved to the farm, the house had been known among the neighbors as an unhealthy one, and there had been much sickness and a number of deaths among its former tenants.

"The house is a frame one, and one of the neighbors said to me that it was an old house when he came to the neighborhood thirty-seven years ago. It consists of two rooms on the ground-floor, with attic rooms above. The frame rests upon four large logs or sills, which lie directly upon the ground, and are thoroughly rotten. There is no cellar under any part of the house. From the front, at least, the surface slopes toward the house, and the rain-water runs under it. In the floor of one room a trap-door had been placed, and directly under this a small excavation had been made for the purpose of collecting the rain-water when it accumulated under the house. Although this pit was dry at the time of our examination, its sides and bottom were marked with cray-fish holes, showing that water had stood in it. The floor was laid of unjointed boards, and every time that it was swept much of the filth fell through the cracks, and every time that the tidy housewife scoured and mopped the floor, the water, carrying with it the filth, ran through the crevices, and thus the conditions most favorable for putrefactive changes were brought into existence and maintained.

"One corner of one of the rooms had been transformed into a small room, or buttery, as it was called, and in this, on shelves, the food was kept. On account of the more frequent scouring demanded by that part of the floor enclosed in this buttery, the boards had rotted away, and a second layer of boards had been placed over the original floor. Between these two floors we found a great mass of moist, decomposing matter, the accumulations of years, which the broom could not reach. When this floor was taken up, a peculiar, nauseating odor was observable, and was sufficient to produce nausea and vomiting in one of the persons engaged in the examination. Some of the dirt from beneath the floor, and some of that which had accumulated beneath the boards in the buttery, were taken for further study.

"The condition of the house was supposed to be unfavorable to the patients, and for this reason they were moved, as Dr. Mesic has stated, to the house of a neighbor. Of course, thorough examination of the house was not made until the patients had been removed.

"Special inquiry was now made concerning the food used by this family. They had been living very simply. They lived upon bread, butter, milk, and potatoes, with coffee and ripe fruit. They had eaten no canned foods for months. They ate but little meat. Occasionally a chicken was killed and served, and rarely, some fresh meat was obtained from the village. During the week in which they were taken ill, all the meat used consisted of slices from a piece of bacon, the only meat which was kept in the house, and a chicken. None of the latter remained, but the bacon was examined. It seemed in perfect condition, and contained no trichinæ. Moreover, as has been seen from the history of the cases, all the members of the family were not made sick by any one meal, but the opportunity of obtaining the poison must have been present for some time. Moreover, the fact that previous similar, but less severe, attacks had occurred at intervals for the past three years, convinced us that the poison must owe its origin to some long-existing condition.

"The drinking-water supply was also investigated. The water was obtained from a shallow well, and some of it was taken for analysis. But several families had for years used water from this well, and had remained healthy.

"The milk used by the family was studied. Of course, we could get none of that which had been used before the members of the family were stricken down. As soon as he made the diagnosis of tyrotoxicon poisoning, Dr. Mesic ordered the discontinuance of the use of milk, not only with the sick, but he forbade the daughter, who had returned, and any of the visitors using it. Mr. Evans owned four milch cows, and they were supplied with fair pasturage and abundant water. The greater part of the milk was placed in tin cans which were set in a wooden trough in the yard, and surrounded by cold water. The covers to the cans were arranged so that the air could have free access to the milk, and were left in this position until the milk was thoroughly cooled. Indeed, the cans were furnished by a creamery company, which followed the directions which I have previously given for the care of milk. On his first visit to me, Dr. Mesic brought some of the milk from one of these cans. This I examined, but failed to find tyrotoxicon in it.

"However, the family did not drink any of the milk from the cans. That which they did use was kept in the buttery which I have described. Here it stood upon a shelf, and some members of the family, at least, were in the habit of drinking from it between meals. This was especially true, it is said, of the son. He would frequently come from his work in the fields, go into the buttery and drink a glass or more of the milk. Mr. Evans states that he frequently observed that the taste of the milk was not pleasant. On my first visit to the premises I advised that some of the milk should be taken from the cans, allowed to stand in the buttery over night, and be sent to me the next day. This was done, and in this milk we found tyrotoxicon, not only by the employment of chemical tests, but by poisoning a kitten with it.

"On the death of the mother and son, Dr. Mesic asked for a post-mortem, but the friends objected, and the undertaker used an arsenical embalming fluid, so that, although consent was subsequently obtained, it was decided that the examination would be so vitiated as to be worthless. On the death of the daughter, the coroner summoned a jury and held an inquest. The post-mortem was conducted by Dr. George A. Hendricks, in the presence of the jury and several physicians who had been invited. Dr. Hendricks has kindly furnished me with his report, which I present here in full:

"The autopsy was held fifteen hours after death. The abdominal viscera were first examined. The great omentum was small, in normal position, covering the small intestine. The small intestine was moderately distended with flatus. The jejunum was ashy-green in color; the ileum purplish-green. About eighteen inches from the termination of the ileum was found a diverticulum two inches in length. The small intestine contained very little alimentary matter. The vermiform appendix was free, contained some small fecal lumps, and showed no evidence of inflammation. The cæcum, ascending, transverse, and descending colon were empty and their circular fibres were tightly constricted, except at intervals where the intestine was distended with gas. The sigmoid flexure was moderately distended with gas, and the rectum contained small bits of fecal matter. The stomach was somewhat contracted and lay wholly upon the left side of the median line. It contained a few ounces of fluid. Its extremities were ligated and the organ removed. The mucous membrane of the stomach and intestine were not examined until they reached the chemist. The duodenum was distended with flatus. The liver was normal in size and appearance. The gall-bladder contained about one ounce of bile. The spleen was normal. One-half ounce of fluid deeply stained with blood was found in Douglas's cul-de-sac. The uterus, Fallopian tubes, and ovaries were deeply congested. The left ovary was enlarged and presented on its posterior surface a hemorrhagic spot, oval, about one-half line in length, and several other less distinct ones. The right ovary was normal in size and showed numerous Graafian scars. The ureters and bladder were normal; the latter contained a small amount of urine. The peritoneum, pancreas, and kidneys were perfectly normal.

"The thoracic cavity was next opened. The lungs were normal; there was about one-half ounce of free serum in the left pleural cavity; none in the right. Pericardium normal; right auricle in diastole; left auricle and both ventricles in systole.

"The dura mater showed venous congestion; the arachnoid, normal; the pia mater, congested. On the surface of the centrum ovale, small drops of blood oozed from the divided vessels. The large veins of the velum interpositum were distended. Third and fourth ventricles were slightly distended with serous fluid, but the walls were normal. There seemed to be slight softening of the optic thalami. The sub-arachnoid fluid was about twice the normal quantity.

"On examination of the mucous membrane of the stomach and intestine in the presence of the chemist, Prof. A. B. Prescott, nothing abnormal could be found. The membrane was stained with bile, but there was not the slightest redness. The solitary glands were distinct, but not at all inflamed. Peyer's patches were normal.

"It will be seen that there existed no lesion which would account for the death. The venous congestion observed in the brain would follow from failure of the heart.

"Some of the post-mortem appearances bore a striking resemblance to those which I had observed in cats poisoned with tyrotoxicon. This was especially noticeable in the condition of the mucous membrane of the stomach and intestine. Tyrotoxicon produces the symptoms of a gastro-intestinal irritant, but not the lesions. The contraction of the circular fibres of the intestine, which undoubtedly caused the constipation, I had also observed in cats that died from tyrotoxicon poisoning without either vomiting or stool.[1] The action of this poison upon the stomach and intestine must be through the nervous system. Small doses cause both vomiting and purging, while after large doses vomiting may be impossible, and obstinate constipation may exist. Both the vomiting and purging after small doses are undoubtedly due in part to increased activity of the circular fibres of the muscular coats, induced through the nerves; and the inability to vomit, and the constipation, one or both of which may be observed after large doses of the poison, are due to spasm of the same muscles, induced in the same manner.

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[1] Marsh reports a case in which the symptoms resembled very closely those of rapidly perforating typhlitis, but the post-mortem examination showed absolutely no evidence of this disease or of peritonitis. In fact the

"Prof. A. B. Prescott was requested by the coroner to analyze the material for mineral and vegetable poisons. He made analyses of the stomach and part of its contents, and a portion of the liver. His results were wholly negative.

"Novy tested a cold-water extract of the finely divided intestine for ptomaïnes. The fluid, which was acid in reaction, was filtered, then neutralized with sodium bicarbonate, and shaken with ether. The ether, after separation, was removed, and allowed to evaporate spontaneously. The residue was dissolved in water, and extracted again with ether. This ether residue gave the chemical reactions for tyrotoxicon, and a portion of it was administered to a kitten about two months old. Within half an hour after the administration the kitten began to retch, and soon it vomited. Within the next three hours it was noticed to vomit as many as five times. The breathing became rapid and labored. The animal sat with its head down, and seemed greatly prostrated. The pupils were examined, but could not be said to be dilated. There was no purging. The retching and heavy breathing, with evidences of prostration, continued more or less marked for two days, after which the animal slowly improved.

"A quantity of fresh milk was divided into five portions of one quart each, placed in quart bottles which had

only abnormality found in the intestines consisted of the contraction of the circular fibres of the transverse and descending colon. Marsh believes that this was a case of ptomaïne poisoning.

been thoroughly cleansed, and treated in the following manner:

"No. 1 consisted of the milk only, and was employed as a control test.

"No. 2 was mixed with a drachm of vomited matter.

"No. 3 was treated with a portion of the contents of the stomach.

"No. 4 was treated with an aqueous extract of the intestine.

"No. 5 was treated with a small portion of the soil which had been taken from the floor of the buttery, stirred up with water.

"These bottles were placed in an air-bath, and kept at a temperature of from 25° to 30° C. for twenty-four hours. Then each was tested for ptomaïnes. No. 1 yielded no tyrotoxicon, while all of the others contained this poison. The tests were both chemical and physiological. All of the samples yielded a non-poisonous base when treated according to Brieger's method, and the same substance was obtained from perfectly fresh milk. It is most probably formed by the action of the heat and reagents employed in this method. This base was obtained in crystalline form, and several portions of it were administered to kittens without any effect. The further study of this body will be of interest to toxicologists, because it gives many of the general alkaloidal reactions. At first we supposed it to be Brieger's neuridine, and this supposition may still be correct, but, as we obtained it, it gave some reactions which are not given by neuridine. Further investigations will be made on this point.

"Tyrotoxicon was obtained from the filtered milk by two methods: (1) The one which we have previously used, and which consists in neutralizing the filtered milk with sodium bicarbonate, and extracting with ether. That portion of the poison employed in the physiological tests was obtained in this way, and in order to be sure that no poison came from the ether, the extract from the milk to which nothing had been added was given to a kitten, and was found to produce no effect. (2) The filtrate from the milk was heated to 70° C. (158° F.) (tyrotoxicon decomposes at 91° C. (195.8° F.)) for some minutes, and filtered. This filtrate, which was perfectly clear, was treated with a small quantity of nitric acid in order to convert the tyrotoxicon into a nitrate, then pure potassium hydrate in the solid form was added until the solution was strongly alkaline. This solution was concentrated so far as it could be on the water-bath. (The potassium compound of tyrotoxicon is not decomposed below 130° C. (234° F.).) The dark brown residue, after cooling, was examined with the microscope and found to contain the crystalline plates of tyrotoxicon-potassium hydrate, along with the prisms of potassium nitrate. The former was separated from the latter by extraction with absolute alcohol and filtration. The alcohol was evaporated to dryness on the water-bath, and the residue again extracted with absolute alcohol. From this alcoholic solution tyrotoxicon was precipitated with ether. The precipitate was decomposed by adding acetic acid and heating, the tyrotoxicon being broken up into nitrogen and phenol. The phenol was recognized by precipitation with bromine water, and by other well-known tests.

"On October 8th, the coroner's inquest, which had been adjourned after the post-mortem in order to await the results of the analysis, was resumed, and after hearing the testimony in accordance with the above stated facts, the jury returned a verdict of death from poisoning with tyrotoxicon."

CAMMAN reports twenty-three cases of milk poisoning which he attributes to tyrotoxicon, although this poison could not be found in the milk. It may be that the active agent present belongs to the bacterial proteids.

KINNICUTT has isolated tyrotoxicon from milk which had been kept for some hours in an unclean vessel.

POISONOUS ICE-CREAM.—In 1886, VAUGHAN and NOVY obtained tyrotoxicon from a cream which had seriously affected many person at Lawton, Michigan. Vanilla had been used for flavoring, and it was supposed that the ill-effects were due to the flavoring. This belief was strengthened by the fact that a portion of the custard was flavored with lemon, and the lemon cream did not affect any one unpleasantly. Fortunately some of the vanilla extract remained in the bottle from which the flavoring for the ice-cream had been taken, and this was forwarded to the chemists. Each of the experimenters took at first thirty drops of the vanilla extract, and no ill-effects following this, one of them took two teaspoonfuls more, with no results. This proved the non-poisonous nature of the vanilla more satisfactorily than could have been done by a chemical analysis.

Later, it was found that that portion of the custard which had been flavored with lemon was frozen immediately; while that portion which was flavored with vanilla and which proved to be poisonous, was allowed to stand for some hours in a building, which is described as follows by a resident of the village:

"The cream was frozen in the back end of an old wooden building on Main Street. It is surrounded by shade, has no underpinning, and the sills have settled into the ground. There are no eve-troughs, and all the water falling from the roof runs under the building, the streets on two sides having been raised since the construction of the house. The building had been unoccupied for a number of months, consequently had had no ventilation, and what is worse, the back end (where the cream was frozen) was last used as a meat market. The cream which was affected was that portion last frozen; consequently it stood in an atmosphere like that of a privy vault for upward of an hour and a half or two hours before being frozen."

The symptoms observed in these cases are given by Dr. MOFITT as follows:

"About two hours after eating the cream every one was taken with severe vomiting, and after from one to six hours later with purging. The vomit was of a soapy character, and the stools watery and frothy. There was some griping of the stomach and abdomen, with severe occipital headache, excruciating backache, and bone pains all over, especially marked in the extremities. The vomiting lasted from two to three hours, then gradually subsided, and everybody felt stretchy, and yawned in spite of all resistance. The throats of all were œdematous. One or two were stupefied; others were cold and experienced some muscular spasms. A numb feeling, with dizziness and momentary loss of consciousness, was complained of by some. Temperature was normal, and pulse from 90 to 120. Tongue dry and chapped. All were thirsty after the vomiting subsided, and called for cold water, which was allowed in small quantities, with no bad results. After getting out no one of the victims was able to be in the hot sun for several days, and even yet (about ten days after the poisoning) the heat affects myself. I attended twelve persons, besides being sick myself, and all were affected in pretty much the same way. Several complain yet of inability to retain food on the stomach without distressing them. The man who made the cream took a teaspoonful of it, and he vomited the same as those who took a whole dish, but not so often or for so long a time. All are affected with an irresistible desire to sleep, which can scarcely be overcome. Even yet, some of us feel that drowsy condition, with occasional occipital headache."

The tyrotoxicon obtained from this cream was administered to a kitten about two months old. Within ten minutes the cat began to retch and soon it vomited. This retching and vomiting continued for two hours, during which the animal was under observation, and the next morning it was observed that the animal had passed several watery stools. After this, although the kitten could walk about the room, it was unable to retain any food. Several times it was observed to lap a little milk, but on doing so it would immediately begin to retch and vomit. Even cold water produced this effect. This condition continuing, after three days the animal was placed under ether and its abdominal organs examined. Marked inflammation of the stomach was supposed to be indicated by the symptoms, but the examination revealed the stomach and small intestine filled with a frothy, serous fluid, such as had formed a portion of the vomited matter, and the mucous membrane very white and soft. There was not the slightest redness anywhere. The liver and other abdominal organs seemed normal.

A bit of the solid portion of this cream was added to some normal milk, which, by the addition of eggs and sugar, was made into a custard. The custard was allowed to stand for three hours in a warm room, after which it was kept in an ice-box until submitted to chemical analysis. In this tyrotoxicon was also found.

Tyrotoxicon has since been found in some chocolate cream which poisoned persons at Geneva, N. Y., and in lemon cream from Amboy, Ohio.

SCHEARER reports the finding of tyrotoxicon in both vanilla and lemon ice-cream which made many sick at Nugent, Iowa.

ALLABEN reports poisoning with lemon cream, and makes the following interesting statements concerning it:

"I would first say July 4, 5, and 6 were very warm. Monday evening, July 5, the custards were cooked, made from Monday morning's cream and Monday night's milk, boiled in a tin pan that had the bright tin worn off. It was noticed that one pan of cream was not sweet, but thinking it would make no difference, it was used; the freezers were thoroughly cleaned and scalded, and the custards put in the same evening while hot; the cream was frozen Tuesday afternoon, having stood in the freezers since the night before, when the weather was very warm."

No analysis of this cream was made, but the symptoms agree with those of tyrotoxicon poisoning.

WELFORD observed several cases of poisoning from custard flavored with lemon. These custards were tested for mineral poisons, with negative results.

MORROW has put forth the claim that ice-cream poisoning is solely due to artificially prepared vanillin, which is, according to his statement, used instead of vanilla extract, but the facts stated above concerning poisoning with creams in which other flavors had been used contradict this claim. Moreover, GIBSON has shown the utter absurdity of the claim, inasmuch as he calculates from the amount of flavoring ordinarily used in ice-cream, that in order to produce the toxic symptoms observed, the flavoring must be ten times as poisonous as pure strychnine.

BARTLEY suggests that poisonous cream sometimes results from the use in its manufacture of poor or putrid gelatin. This is highly probable, and with the gelatin the germs of putrefaction may be added to the milk.

POISONOUS MEAL AND BREAD.—Reference has already been made to the fact that the peasants in certain parts of Italy are frequently poisoned by eating mouldy corn-meal. As has also been stated, LOMBROSO and others have obtained from this meal ptomaïnes, some of which give the same color reaction as strychnine. In 1886, LADD succeeded in isolating from "heated" corn-meal a ptomaïne which forms in urea-like crystals. The quantity was not sufficient for an ultimate analysis, and the physiological action has not been studied. Poisoning from decomposed and mouldy bread is not unknown.

CHAPTER IV.

GENERAL CONSIDERATIONS OF THE RELATION OF BACTERIAL POISONS TO INFECTIOUS DISEASES.

THE majority of diseases may be grouped from an etiological standpoint into the following classes: (1) Traumatic; (2) infectious; (3) autogenous; and (4) neurotic. It must be understood, however, that in many diseases the cause is not single, but multiple, and for this reason sharp lines of classification cannot be drawn. For instance, the greatest danger in those traumatic affections in which the traumatism itself does not cause death, lies in infection. The wound has simply provided a suitable point of entrance for the infecting agent. Indeed, the break in the continuity of tissue may be so slight that it is of import and danger only on account of the coincident infection. This is true in many cases of tetanus. Furthermore, an infectious disease, whether it originates in a traumatism or not, is markedly influenced by what we are pleased to call the idiosyncrasy of the patient, and by which we mean the peculiarities of tissue metabolism taking place in the individual at the time. A dozen men may be exposed alike to the same infection, and the infecting agent may find a suitable soil for its growth and development in two of these, while in the other ten this same agent meets with such adverse influences that it dies without producing any appreciable effects; or all may be infected, but with difference in degree, as is evidenced by variation in symptoms, in the length of time that this infecting agent continues to grow and develop in the body and in the ultimate result. Every physician who has had experience in the treatment of typhoid fever, diphtheria, scarlet fever, or, in short, of any of the infectious diseases, will appreciate the importance of the personal equation in his patients.

Charrin and **Roger** have shown that white rats, which are naturally immune to anthrax, become susceptible when fatigued by being kept on a small tread-mill. Eleven rats were inoculated with an anthrax culture; five of these which were allowed to rest in the cage manifested no symptoms of the disease, while six which were placed on the tread-mill developed the disease and died within from twenty-four to thirty hours. The bacilli were found in the liver and spleen of those which died; and guinea-pigs inoculated with these germs died. The influence of the condition of health on susceptibility to the infectious diseases has also been shown by **Leo**, who found that mice which are naturally insusceptible to glanders, become highly susceptible when they are rendered diabetic by the administration of phloridzin.

That some neurotic affections originate from traumatism we know. That others of this class are largely due to malnutrition accompanied by improper metabolism or insufficient elimination, or, in other words, are to some extent autogenous, all believe. Understanding, then, that the above classification does not attempt a sharp and marked differentiation of the causes of disease, we will now give our attention to a consideration of the chemical factors in the causation of the infectious diseases, and of the traumatic, autogenous and neurotic, in so far as these are influenced by infection.

Recognizing the fact that germs do bear a causal relation to some diseases, the question arises, How do these organisms produce disease? In what way does the bacillus anthracis, for instance, induce the symptoms of the disease and death? Many answers to this question have been offered. Some of the most important of these are as follows:

1. It was first suggested by **Bollinger** that apoplectiform anthrax is due to deoxidation of the blood by the bacilli. These germs are aërobic, and were supposed to deprive the red blood-corpuscles of their oxygen. This theory was suggested most probably by the resemblance of the symptoms to those of carbonic acid poisoning. The

most prominent of these symptoms are dyspnœa, cyanosis, convulsions, dilated pupils, subnormal temperature, and, in general, the phenomena of asphyxia. Moreover, post-mortem examination reveals conditions similar to those observed after death by deprivation of oxygen. The veins are distended, the blood is dark and thick, the parenchymatous organs are cyanotic, and the lungs hyperæmic. **Bollinger** compared this form of anthrax to poisoning with hydrocyanic acid, which was then believed to produce fatal results by robbing the blood of its oxygen.

This theory was supported by the observations of **Szpilmann**, who found that while the putrefactive bacteria are destroyed by ozone, the bacillus anthracis thrives and multiplies in this gas.

This theory pre-supposed a large number of bacilli in the blood, and this accorded with the estimate of **Davaine**, which placed the number at from eight to ten million in a single drop. But more extended and careful observation showed that the blood of animals dead from anthrax is often very poor in bacilli. **Virchow** reported cases of this kind. **Bollinger** himself found the bacilli often confined to certain organs and not abundant in the blood. Then **Siedamgrotzky** counted the organisms in the blood in various cases and found not only that the estimate made by **Davaine** is too large, but that in many instances the number present in the blood is small. **Joffroy** found in some of his inoculation experiments that the animals died before any bacilli appeared in the blood. These and other investigations of similar character began to cause workers in this field of research to doubt the truth of the theory of **Bollinger**, and these doubts were soon converted into positive evidence against it. **Pasteur**, in support of the theory, reported that birds were not susceptible to anthrax, and he accounted for this by supposing that the blood corpuscles in birds do not part with their oxygen readily. However, it was shown by **Oemler** and **Feser** that the learned Frenchman had generalized from limited data, and that many birds are especially susceptible to the disease. **Oemler** found that the blood even when rich in bacilli still possesses the bright-red color of oxy-hæmoglobin. **Toepper** and **Roloff** reported cases of apoplectiform anthrax in which there was no difficulty in respiration. **Toussaint** caused animals which had been inoculated with the anthrax bacillus to breathe air containing a large volume of oxygen, and found that this did not modify the symptoms or retard death. Finally, **Nencki** determined the amount of physiological oxidation going on in the bodies of animals sick with anthrax by estimating the amount of phenol excreted after the administration of one gramme of benzol, and found that the oxidation of the benzol was not diminished by the disease. Thus, the theory that germs destroy life by depriving the blood of its oxygen has been found not to be true for anthrax, and if not true for anthrax, certainly it cannot be for any other known disease. The bacillus anthracis is, as has been stated, aërobic, while most of the pathogenic bacteria are anaërobic—that is, they live in the absence of oxygen. This element is not necessary to their existence, and, indeed, when present in large amount, it is fatal to them. Moreover, in many diseases, the bacteria are not found in the blood at all. Lastly, the symptoms of these diseases are not those of asphyxia. These facts have caused all bacteriologists to acknowlege that this theory is not the right one.

2. If a properly stained section of a kidney taken from a guinea-pig, which has been inoculated with the bacillus anthracis, be examined under a microscope, the bacilli will be found to be present in such large numbers that they form emboli, which not only close, but actually distend the capillaries and larger bloodvessels, and interfere with the normal functions of the organ. A similar condition is sometimes found on microscopical examination of the liver, spleen, and lungs. From these appearances, it was inferred by **Bollinger** that the bacilli produce the diseased condition simply by accumulating in large numbers in these important organs, and mechanically interrupting their functions. This is known as the mechanical interference theory.

Klebs and **Toussaint** were formerly ardent advocates of this theory in its application to anthrax, and the latter thought that the symptoms and death are due to stoppage of the pulmonary circulation by means of emboli. However, HOFFA studied this point by making numerous post-mortem examinations, and was unable to confirm it. A like result followed the work of VIRCHOW, COLIN, and SIEDAMGROTZKY, and the mechanical-interference theory has been abandoned.

In the majority of germ diseases this theory never had any support. There is not found any great accumulation of bacteria in any organ, and the number and distribution of the germs are such that the theory of mechanical interference cannot be held.

3. Another answer given to the question, How do germs cause disease? is, that they do so by consuming the proteids of the body and thus deprive it of its sustenance. The proteids are known to be necessary for the building up of cells, and it is also known that microörganisms feed upon proteids. But this theory is untenable for several reasons. In the first place, many of the infectious diseases destroy life so quickly that the fatal effect cannot be supposed to be due to the consumption of any very large amount of proteids. In the second place, the distribution of the microorganisms is such in many diseases that they do not come in contact with any large proportion of the proteids of the body. In the third place, the symptoms of the majority of these diseases are not those which would be produced by withdrawing from the various organs their food. The symptoms are not those of general starvation.

4. Still another theory, which has been offered, is that the bacteria destroy the blood corpuscles, or lead to their rapid disintegration. But in many of the infectious diseases, as has been stated, the microörganisms, although very abundant in some organs, are not present in the blood. Moreover, the disintegration of the blood corpuscles is not confirmed by microscopical examination.

5. Seeing the vital deficiencies in the above theories, and being impressed by the results obtained by the chemical study of putrefaction, bacteriologists have been led to inquire into the possibility of the symptoms of the infectious

diseases being due to chemical poisons. In investigating this theory, three possibilities suggest themselves:

(*a*) The microörganisms themselves may be poisonous, or the poison may be an integral part of them. **Neelsen**, at one time an advocate of this theory, thus accounted for the appearance and increase in violence of the symptoms as the germs increase in number. In order for the conditions of this theory to be fulfilled the microörganisms must be present in the blood before any of the symptoms appear. But in anthrax the most thoroughly studied of all the infectious diseases, and the one to which all these theories have been applied, the bacilli first appear in the blood, as a rule, only a few hours before death, and long after the appearance of the first symptoms; while in many other diseases the germs are never found in the blood. Moreover, as **Hoffa** has shown, if this theory be true, the injection of a large quantity of anthrax bacilli directly into the blood should be followed immediately by symptoms of the disease, and death should be speedy. But he found, on making experiments of this kind, that the symptoms did not appear until from twenty-four to seventy-two hours. **Nencki** found by analysis that the substance of the anthrax bacilli resembles vegetable casein in some respects, and animal mucin in others. This "anthrax protein" is freely soluble in alkalies, is insoluble in water, acetic acid, and the dilute mineral acids. It contains no sulphur and was believed by **Nencki** to be inert; but the recent researches of **Buchner** has shown that this belief is not well founded. It has been stated by a number of investigators that suppuration might be induced by the injection of certain sterilized cultures, but the dictum of **Weigert**, "no suppuration without bacteria," has been generally accepted; and statements to the contrary, although some of them have been made by men of excellent reputation, have until recently received but little credence. However, **Buchner** has shown conclusively that the albuminate of the bacterial cell in as many as seventeen different species possesses well-marked pyogenetic properties, and that the pus formed is free from germs. **Buchner** separated the microörganisms from the soluble substances accompanying them by sedimentation and decantation, washed the cells, dissolved them in a 0.5 per cent. solution of potash by the aid of heat, precipitated the albumin with dilute mineral acid, and, after repeated resolution in alkali and reprecipitation with acid, employed the purified proteid in his experiments. Introduced with antiseptic precautions under the skin, this substance invariably causes suppuration. This demonstrates that the substance of the bacterial cell is not altogether inert. It is impossible at present to say to what extent the course of an infectious disease may be influenced by the breaking down of a large number of bacterial cells and the introduction of their substance into the blood.

(*b*) The microörganisms may be intimately associated with or may produce a soluble, chemical ferment, which, by its action on the body, produces the symptoms of the disease and death. This theory formerly had a number of ardent supporters, among whom might be mentioned the eminent scientist, DE BARY. But PASTEUR proved the theory false when he filtered anthrax blood through earthen cylinders, inoculated animals with the filtrate, and failed to produce any effect. NENCKI made a similar demonstration when he inoculated a two per cent. gelatin preparation with the anthrax bacillus, which liquefied the preparation, and on standing the bacilli settled to the bottom. The supernatant fluid, which was clear, alkaline in reaction, and contained dissolved "anthraxprotein," was filtered and injected into animals without producing any effect.[1]

It must not be inferred from the above statements that bacteria do not produce any ferments. Many of them do form both diastatic and peptic ferments, which may retain their activity after the bacteria have been destroyed; but there is no proof that in any case these ferments have any causal relation to the disease. After the diseased process has been inaugurated some of these ferments probably play an important part in the production of morphological changes, the nature of which will be indicated when these diseases are discussed.

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[1] We now know that if the supernatant fluid used in this experiment had been injected in sufficient quantity death would have been produced by the soluble chemical poisons.

(c) The germ may produce chemical poisons by splitting up preëxisting complex compounds in the body. This theory finds, in the first place, strong support in the well-known fact that many of the putrefactive germs produce highly poisonous bodies; and, in the second place, the formation of chemical poisons will account for the appearance of the symptoms of the disease when the microörganisms never find their way into the blood. The correctness of this theory has been tested by a large number of investigators, and with the result that its truth has been firmly established. It was soon found that pathogenic germs grown in meat broth and other culture media elaborate chemical poisons which, when injected into the lower animals, induce in an acute form one or more of the symptoms characteristic of the disease caused in man by the microörganism. It is true that until quite recently this theory has been opposed by some, and it is altogether possible that at present there may be those who are not altogether convinced of its truth. However, we are not acquainted with any argument against it which remains unanswered. For a while BAUMGARTEN claimed that the formation of chemical poisons in the dead matter of meat broth and other media by the germ does not prove that the same agent is capable of forming the same or similar products within the living body; but the isolation of tetanine from the amputated arm of a man with tetanus, by BRIEGER, furnished the first positive answer to this criticism, and since that time a number of bacterial poisons have been obtained from the bodies of men and the lower animals. We now expect to find each specific, pathogenic microörganism producing its characteristic poison or poisons. The evidence on this point will be given further on in a brief sketch of the chemical factors in the causation of some of the best-known infectious diseases.

Before taking up the individual diseases, we will give what appears to us, in the present state of our knowledge, a correct definition of an infectious disease.

An infectious disease arises when a specific, pathogenic microörganism, having gained admittance to the body, and having found the conditions favorable, grows and multiplies, and in so doing elaborates a chemical poison which induces its characteristic effects.

In the systemic infectious diseases, such as anthrax, typhoid fever, and cholera, this poison is undoubtedly taken into the general circulation, and affects the central nervous system. In the local infectious diseases, such as gonorrhœa, and infectious ophthalmia, the principal action of the poison seems to be confined to the place of its formation. Though even in these, when of a specially virulent type, the effects may extend to the general health. It may be that in some diseases the chemical poison has both a local and a systemic effect. Thus, it is by no means certain that the ulceration of typhoid fever is due directly to the bacillus. On the other hand, it is altogether probable that the anatomical changes in the intestine result from the irritating effects of the poison at the place of its formation.

With the proof, that the deleterious effects wrought by germs are due to chemical poisons elaborated by them during their growth, admitted, let us inquire what properties a microörganism must possess before it can be said to be the *specific* cause of a disease. The four rules of KOCH have been generally conceded to be sufficient to show that a given germ is the *sole* and sufficient cause of the disease with which that germ is associated. Briefly, these rules are as follows:

1. The germ must be present in all cases of that disease.
2. The germ must be isolated from other organisms and from all other matter found with it in the diseased animal.
3. The germ thus freed from all foreign matter must, when properly introduced, produce the disease in healthy animals.
4. The microörganism must be found properly dis-

tributed in the animal in which the disease has been induced.

Let us give our special attention to the first of these rules for a few moments. What is meant by the statement that the special germ must be found in every case of the disease? How will A, pursuing his studies on the bacteriology of a given disease in America, decide whether or not a bacillus which he finds is identical with one which has been reported as invariably present in the same disease by B, who has investigated an epidemic in Germany? What means are relied upon to prove the identity of these two organisms? The means which have been relied upon wholly are the form, size, reaction with staining reagents, manner of growth on various nutrient media, and, in exceptional instances, correspondence in their effects upon the lower animals. In other words, with the exception of those instances in which the effects upon animals are tried, the characteristic property by which the germ causes the disease is left wholly out of consideration. It is admitted that any causal relation which the germ may have to the disease is due to its capability of forming one or more chemical poisons, and yet no attempt is made to ascertain whether or not it possesses this property. Indeed, some of the most eminent bacteriologists have taught that in the identification of germs the reactions with staining reagents and the appearance of the growths on the various nutritive media are of more importance than the observation of the effects upon animals. Thus, FLÜGGE says:

"Inoculation experiments with both typhoid dejections and pure cultures of the Eberth bacillus have universally been without success. The few experiments in which a typhoid disease has followed inoculation or feeding have been made with impure material containing other active bacteria. It is known that a group of widely distributed organisms, which, however, are wholly different from the typhoid bacillus, have the power, when injected subcutaneously or intravenously, of producing in animals death with marked swelling and ulceration of Peyer's patches. To these organisms undoubtedly are due the apparently positive results which some authors have supposed to be due to inoculation with the typhoid bacillus."

In other words, this eminent author teaches that although other germs may cause the essential symptoms and lesions of typhoid fever in the lower animals, they are not related to the germ found in the spleen of man after death from typhoid fever, because they do not react in the same manner with the anilin stains, and present a different appearance in their growths on potatoes.

We will suppose that in an epidemic of diphtheria, A examines the membrane from a hundred, or we might as well suppose a thousand, children, and finds a characteristic, well-marked, easily recognized bacillus in all. He isolates this organism, and obtains it in pure culture. He inoculates animals, and these manifest all the signs, together with the appearance of the characteristic membrane of diphtheria, and in these animals he finds his bacillus growing as in the throats of the children. All the rules of KOCH have been complied with. Has A demonstrated that his bacillus is the *sole* cause of diphtheria? No. He has shown that his bacillus is *a* cause of diphtheria; but he has not proven that there may not be other germs, wholly different from his in form and size, which may also cause diphtheria. The most which can be proven by KOCH's rules is that a given germ is *a* cause of a certain disease. They do not show, as most bacteriologists would have us believe, that the given germ is the *sole* cause of the disease.

To illustrate, we will suppose that a botanist in visiting Arabia should find a tree producing a berry, the coffee berry, which, when properly prepared and taken into the system, produces certain effects which are due to the alkaloid, caffein, and which invariably follow the drinking of a decoction of these berries; would our supposed discoverer be justified in concluding that the coffee tree is the only plant in the world capable of producing these supposed characteristic effects? Should he reach such a conclusion, the fact that it is not warranted would be shown by a study of the tea plant of China and the guarana of South America.

The moment that it is granted that the real poison of the disease is chemical in character, it becomes evident that no one is justified in saying that one germ is the sole source of that poison. Such a statement would be as unwarranted as one that the coffee tree is the sole source of caffein, or that the strychnos Ignatii is the only species of the natural order Loganiaceæ which produces a convulsive poison. In other words, the specific cause of a given disease is not to be determined wholly by the morphology of the germ, but by the character of the chemical poison which is the true materies morbi.

Bacteria cannot be classified, so far as their causal relationship to disease is concerned (and this is the most important knowledge to be gained from them), until we know the nature of their chemical products, for it is by virtue of these that the germs have any causal relationship to disease.

It is possible that two germs may be unlike in form, and yet they may produce poisons which are identical or those which are very similar in their effects upon man. One germ may be stained by GRAM's method and another fail to be acted upon when so treated; but this does not prove that their chemical products are totally unlike. This is not only a possibility, it is a fact which has been demonstrated repeatedly, both with pathogenic and non-pathogenic organisms. A few illustrations may be given here: The yeast plant is not the only microörganism which will produce alcohol in saccharine solutions. The same product results from the growth of the bacterium Bischleri, bacterium coli commune, bacterium ilei, bacterium ovale ilei, bacterium lactis aërogenes, and others (NENCKI). Morphologically, there are marked differences between the yeast plant and these bacteria, but they alike produce alcohol. More than a dozen germs, including both micrococci and bacilli, are capable of generating lactic acid. Some of these produce an acid which is optically inactive; others, one which is dextro-rotatory; and others still, one which is lævo-rotatory. The tetanus germ of KITASATO and that of TIZZONI and CANTANI are known to be different. Cultures of the former in bouillon are virulent, while those of the latter in the same medium are inert. Not only are these two organisms morphologically and biologically distinct, but their poisons are chemically unlike. BRIEGER and FRÄNKEL precipitated the poisonous albumin of the germ of KITASATO with alcohol, but this reagent renders the poison of the Italian germ inert. Notwithstanding this difference, however, both microörganisms and their chemical products produce tetanic convulsions and death in the lower animals. We must, therefore, admit that there are at least two distinct germs, each of which is capable of causing tetanus; and how many other bacteria with like properties there may be no one can tell. All attempts to find a morphologically specific germ in the summer diarrhœas of infancy have failed. The labors of BOOKER in this country and of ESCHERICH in Germany have shown that no one species or variety is constantly present. No less than thirty distinct germs have been obtained from the bowels and feces of children suffering from these diarrhœas. A germ which is frequently present one season may not be found at all the next. Are we to conclude from this failure to comply with the first of KOCH'S rules, that the summer diarrhœas of infancy are not due to microörganisms? Certainly not; especially in view of the fact that BAGINSKY and STADTHAGEN have obtained from pure cultures of a saprophytic germ found in the stools of cholera infantum a poisonous base and a poisonous proteid; and VAUGHAN has shown that at least three of BOOKER'S bacteria produce chemical poisons which cause in kittens retching, vomiting, purging, collapse, and death. To the contrary we are justified in concluding that these diarrhœas may be due to any one or more of a number of germs which differ from one another sufficiently morphologically to be classified as distinct species. The similarity among these bacteria will not be discovered by a study of their size, form, and reactions with staining agents, but by a study of their chemical products, the agents by virtue of which they cause the disease.

We think that we are justified in concluding that in those diseases in which the four rules of KOCH have been complied with, the germ is *a* cause of the disease, but our range of observation must be much wider than it now is before we can say that the given germ is the *only* cause of the disease.

We believe that those few infectious diseases, such as anthrax and tuberculosis, which have such well-marked, typical, clinical histories, are due to equally well-marked and morphologically distinct microörganisms which can be recognized by microscopical study alone; but we do not believe that this is true in diseases showing such wide variations in symptoms as is the case in typhoid fever and cholera infantum.

In all cases, we insist that the true test of the specific character of a germ is to be made with its chemical products. A given bacterium may not multiply in the circulating blood of a dog, and failure to do so is by no means proof that the same organism might not cause disease in man; but every germ which causes disease in man does so by virtue of its chemical products, and if these be isolated and injected into the dog in sufficient quantity a poisonous effect will be produced. In the study of the bacteriology of the infectious diseases, the third and fourth of KOCH's rules have not been complied with in many diseases on account of the insusceptibility of the lower animals. The majority of investigators, meeting with this difficulty, have been inclined to rest content with the first two rules, and to conclude that when a given germ is constantly present in a given disease, and not found in other diseases, that it is the cause of the disease with which it is associated. Indeed, we find so good an authority as WELCH stating that the successful inoculation of animals is not necessary in order to prove the causal relationship of a germ to a disease. In 1889, VAUGHAN suggested that in those instances in which the third and fourth of KOCH's rules cannot be complied with on account of the insusceptibility of the lower animals, it must be shown that the germ can produce chemical poisons which will induce in the lower animals in an acute form the characteristic symptoms of the disease, before the proof that the given germ is the cause of the disease be accepted as positive.

Heretofore, the science of bacteriology has been largely founded upon morphological studies. Bacteriologists have given their time and attention to the discovery of bacterial forms in the diseased organism and to observations of characteristics in structure and growth of different species of bacterial life. We must now study the physiology and chemistry of the germs, and until this is done we must remain ignorant of the true cause of disease, and so long as we remain ignorant of the cause, it cannot be expected that we shall discover scientific and successful methods of treatment. Suppose that our knowledge of the yeast plant was limited to its form and method of growth; of how little practical importance this knowledge would be. That the yeast plant requires a saccharine soil before it can grow, that given such a soil it produces carbonic acid gas and alcohol, are the most important and practical facts which have been ascertained in its study. Likewise, the conditions under which pathogenic germs multiply and the products which they elaborate in their multiplication must be ascertained before their true relationship to disease can be understood.

In saying that the morphological work upon which the science of bacteriology rests almost wholly is inadequate, we wish that it may be plainly understood that we are not offering any hostile criticism upon the great men who have done this work and who have formulated conclusions therefrom. The development of bacteriology has been in accordance with the natural law governing the growth of all the biological sciences. The study of form naturally and necessarily precedes the study of function. The ornithologist finds a new species of bird. He first studies its shape and size, the color of its plumage, the form of its beak, the number and arrangement of the feathers of the tail and wing, the color of the eyes, etc. All this he can do with a single specimen, recognizing the fact, however, that variations more or less marked are likely to be found in other individuals. More time and wider opportunities of observation will be needed before he can tell where and when this bird is accustomed to build its nest, upon what insects, grains, and berries it feeds, with what other species of birds it lives in peace and with what it is at war. A much greater range of observation and study is necessary before the naturalist can tell how his newly discovered species would thrive if carried to a new climate, where it would be compelled to live upon unaccustomed food, to build its nest of strange material, and to encounter new foes.

We repeat that it is no discredit to the science nor to the men who have developed it to say that the study of bacteriology has hitherto been almost wholly morphological. Without the morphologist the physiologist and the physiological chemist could not exist. The science having had for its support only morphological studies, the deductions and formulated statements arrived at by its students have been reached in accordance with the knowledge obtained from this source. But now, it being admitted that the causal relation between a given germ and a certain disease is dependent upon the chemical products of the growth of the germ, the fundamental lines of work must be altered in order to correspond with this new knowledge.

The study of the chemical factors in the causation of the infectious diseases opens up for us a field in which much work must be done. Let us attempt a statement of the nature of some of the researches that must be carried out along this line.

In the first place, we must ascertain what germs are toxicogenic. This would necessitate a chemical study of all kinds of bacteria, both the pathogenic and the non-pathogenic. Every fact ascertained in this investigation will not have its practical application in medicine, but will have its scientific value, and many will most probably be of more or less direct service to man.

Secondly, it must be determined under what conditions these germs are toxicogenic. It is not at all probable that all those bacteria which are capable of producing poisons when grown on dead material outside of the body are also capable of multiplication and the production of the same substances when under the influence of the various secretions of the body. Some bacteria are destroyed by a normal gastric juice within a short time, while others are not. The conditions of life and growth are different when the infecting agent is introduced into the blood from what they are when infection occurs by the way of the alimentary canal. This is well recognized in the two forms of anthrax, one of which arises from inoculation through a wound and the other by way of the intestines. A preventive treatment which is efficient in one is of no service in the other. Then, again, we are to study those conditions of the blood and other fluids of the body which are especially unfavorable to the successful implantation or the continued existence of an infectious disease.

Thirdly, the chemical properties and the physiological action of these poisons will demand careful attention. Some are especially depressing in their action upon the heart, others seem to manifest their chief energy upon the central nervous system, while others still act like true gastro-intestinal irritants. In the study of the toxicological effects of these bacterial poisons every method of investigation known in the most advanced physiological work must be employed. The action of these agents on the heart, the brain, the spinal cord, etc., must be thoroughly studied.

CHAPTER V.

THE BACTERIAL POISONS OF SOME OF THE INFECTIOUS DISEASES.

WE will now give our attention to the chemical poisons, both the ptomaïnes and the proteids, of some of the infectious diseases, and in doing this we will illustrate and substantiate the statements made in the preceding chapter.

ANTHRAX.—The definition of an infectious disease, as we have given it, is well illustrated by the facts which have been learned concerning the causation of anthrax, which has probably been more thoroughly studied than any other infectious disease. KAUSCH taught that this disease has its origin in paralysis of the nerves of respiration. As to the cause of this paralysis he gave us no information. DELAFOND thought that anthrax has its origin in the influence of the chemical composition of the soil affecting the food of animals and leading to abnormal nutrition. The investigations of GERLACH in 1845 demonstrated the contagious nature of the disease, which was emphasized by HEUSINGER in 1850 and accepted by VIRCHOW in 1855. However, as early as 1849, POLLENDER found numerous rod-like micro-organisms in the blood of animals with the disease. This observation was confirmed by BRAUELL, who produced the disease in healthy animals by inoculations with matter taken from a pustule on a sick horse. Attempts were made to ridicule the idea that these germs might be the cause of the disease, and it was said that the bodies seen were only fine shreds of fibrin or blood crystals. Some claimed that the rod-like organisms reported were due to defects in the glass, while others claimed that the defects existed in the eye of the observer, and others still suggested that the defects might be found back of the eye and in the brain. But in 1863, **Davaine** showed that these little bodies must have some causal relation to the disease, inasmuch as his experiments proved that inoculation of healthy animals with the blood of those sick with anthrax produced the disease only when taken at a time when the blood contained these organisms. He also demonstrated beyond any question that these rod-like bodies are bacteria, capable of growth and multiplication. The conclusions of this investigator were combated by many; but **Pasteur**, **Koch**, **Bollinger**, **de Barry**, and others, studied the morphology and life-history of these organisms, and then came the brilliant results of **Pasteur** and **Koch** in securing protection against inoculation anthrax by the vaccination of healthy animals with the modified germ and subsequent inoculation with the virulent form. Now, the bacillus anthracis is known in every bacteriological laboratory, and by inoculation with it the disease is communicated at will to susceptible animals. But here the question arose, How do these bacilli produce anthrax? and in answer to this question the various theories which we have mentioned were proposed.

The first successful attempt to study the chemical poisons of anthrax was made by **Hoffa**, who obtained from pure cultures of the bacillus small quantities of a ptomaïne, which, when injected under the skin of animals, produces the symptoms of the disease and death. This substance causes at first increased respiration and action of the heart, then the respirations become deep, slow, and irregular; the temperature falls below the normal; the pupils are dilated, and a bloody diarrhœa sets in. On section the heart is found contracted, the blood dark, and ecchymoses are observed on the pericardium and peritoneum. **Hoffa** names his poison anthracin. Recently **Hoffa** has isolated this poison from the bodies of animals dead from anthrax.

It has been said that **Hoffa**'s work was the first successful attempt to study the chemical poisons of anthrax. However, his results cannot be considered altogether satisfactory. The small amount of the basic substance which he obtained rendered it highly probable that in the case of a germ so virulent as that of anthrax there must be other chemical poisons produced. This supposition has been confirmed by the labors of HANKIN, who, in 1889, while at work in KOCH'S laboratory, prepared from cultures of the bacillus anthracis an albumose which, when employed in comparatively large amount, proved fatal to animals, but when used in very small quantity gave immunity against subsequent inoculations with the living germ. Unfortunately, HANKIN does not mention the symptoms induced by toxical doses of this substance. Whether or not the albumose of HANKIN contains *in statu nascendi* the base of HOFFA, and owes its poisonous properties to the same, has not been determined.

BRIEGER and FRÄNKEL obtained the poisonous proteid of anthrax from animals in which the disease had been induced by inoculation with the bacillus. The liver, spleen, lungs, and kidneys of these animals were finely divided and rubbed up with water. After this had stood in a refrigerator for twelve hours it was passed through a Chamberland filter and the proteid precipitated from the filtrate with absolute alcohol.

MARTIN, by growing the anthrax bacillus for from ten to fifteen days in an alkaline albuminate from blood serum and filtration through porcelain, obtains the following metabolic products:

1. Protoalbumose and deuteroalbumose and a trace of peptone. All of these react chemically like similar substances prepared by peptic digestion.
2. An alkaloid.
3. Small quantities of leucin and tyrosin.

The most characteristic property of the albumoses is that their solutions are strongly alkaline, and the alkalinity is not removed by treatment with alcohol, benzol, chloroform, or ether, or by dialysis.

The alkaloid is soluble in water, alcohol, and amylic alcohol; insoluble in chloroform, ether, and benzol. Its solutions are strongly alkaline and the alkaloid forms crystalline salts with acids. It is precipitated by the general alkaloidal reagents, with the exception of potassio-mercuric iodide. It is somewhat volatile and loses its poisonous properties on exposure to the air.

The mixed albumoses are poisonous only in considerable doses, 0.3 gramme being required to kill a mouse of 22 grammes weight when injected subcutaneously. Smaller doses cause a local œdema and a somnolent condition, from which the animal recovers. The larger doses produce a more extensive œdema and the somnolence deepens into coma, terminating in death. In some cases the spleen is enlarged. The absence of germs was demonstrated by plate cultures. The alkaloid causes similar symptoms. It is, however, more poisonous and acts more rapidly than the albumoses. The animal is affected immediately after the injection, and the gradually increasing coma terminates in death. The alkaloid also produces œdema, and in many cases thrombosis of the small veins. Extravasation into the peritoneal cavity is occasionally seen, and the spleen is ordinarily enlarged and filled with blood. The fatal dose for a mouse is from 0.1 to 0.15 gramme, death resulting within three hours.

This alkaloid does not appear to be identical in its action with the anthracin of HOFFA.

ASIATIC CHOLERA.—There are good reasons, apart from experimental evidence, for believing that the comma bacillus of KOCH produces its ill effects by the elaboration of chemical poisons. This germ is not a blood parasite. It grows only in the intestine, and the symptoms of the disease and death must result from the absorption of its poisonous products. In confirmation of this statement experiment has shown that this is one of the most active, chemically, of all known pathogenic germs.

In the first place, BITTER has shown that the comma bacillus produces in meat-peptone cultures a peptonizing ferment, which remains active after the organism has been destroyed. Like similar chemical ferments, it converts an indefinite amount of coagulated albumin into peptone. It is more active in alkaline than in acid solutions, thus resembling pancreatin more than pepsin. This resemblance to pancreatin is further demonstrated by the fact that its activity is increased by the presence of certain chemicals, such as sodium carbonate and sodium salicylate. That a diastatic ferment is also produced by the growth of the bacillus was indicated in the experiments of BITTER by the development of an acid in nutrient solutions containing starch paste. However, all attempts to isolate the diastatic ferment were unsuccessful. A temperature of 60° destroys or greatly decreases the activity of ptyalin, and this seems to be true also of the diastatic ferment produced by the comma bacillus. But the formation of an acid from the starch pre-supposes that the starch is first converted into a soluble form.

FERMI has succeeded in isolating the peptonizing ferment of the cholera germ in the following manner: 65 per cent. alcohol added to gelatin which has been liquefied by the bacillus precipitates the proteid, but not the ferment. After twenty-four hours the precipitate is removed by filtration and the ferment precipitated from the filtrate by the addition of absolute alcohol. After being collected on a filter and dried the ferment is dissolved in an aqueous solution of thymol and its peptonizing properties demonstrated on gelatin tubes.

RIETSCH believes that the destructive changes observed in the intestines in cholera are due to the action of the peptonizing ferment.

CANTANI injected sterilized cultures of the comma bacillus into the peritoneal cavities of small dogs and observed after from one-quarter to one-half hour the following symptoms: Great weakness, tremor of the muscles, drooping of the head, prostration, convulsive contractions of the posterior extremities, repeated vomiting, and cold head and extremities. After two hours these symptoms began to abate, and after twenty-four hours recovery seemed complete. Control experiments with the same amounts of uninfected beef-tea were made with negative results. The cultures used were three days old when sterilized. Older cultures seemed less poisonous and a high or prolonged heat in sterilization decreased the toxicity of the fluid. From these facts **Cantani** concluded that the poisonous principle is volatile, but the effect of high or prolonged heat in diminishing the toxicity was more probably due to its destructive effect on the poisonous proteids.

Cantani also observed that the blood of those sick with cholera is acid: this has been confirmed by **Strauss** by the examination of the blood directly after death; and **Ahrend** found lactic acid in the strongly acid urine of a cholera patient.

Nicati and **Rietsch** produced fatal effects in dogs by injecting cultures, from which all germs had been removed by filtration, into the bloodvessels. Later, the same investigators obtained from old bouillon cultures containing peptone a poisonous base. **Ermengen** also showed that cultures after filtration through a Chamberland filter are poisonous.

Klebs has attemped to answer experimentally the question, In what way does the cholera germ prove harmful? Cultures of the bacillus in fish preparations were acidified, filtered, the filtrate evaporated on the water-bath, the residue taken up with alcohol and precipitated with platinum chloride. The platinum was removed with hydrogen sulphide, and the crystalline residue obtained on evaporation was dissolved in water and injected intravenously into rabbits. Muscular contractions were induced. Death followed in one animal, which, in addition to the above treatment, received an injection of a non-sterilized culture. In this case there was observed an extensive calcification of the epithelium of the uriniferous tubules. **Klebs** believes this change in the kidney to be induced by the chemical poison, and from this standpoint he explains the symptoms of cholera as follows: The cyanosis is a consequence of arterial contraction, the first effect of the poison. The muscular contractions also result from the action of the poison. The serous exudate into the intestines follows upon epithelial necrosis. Anuria and the subsequent symptoms appear when the formation and absorption of the poison become greatest.

HUEPPE states that the severe symptoms of cholera can be explained only on the supposition that the bacilli produce a chemical poison, and that this poison resembles muscarine in its action.

VILLIERS isolated by the STAS-OTTO method from two bodies dead from cholera, a poisonous base which was liquid, pungent to the taste, and possessed the odor of hawthorn. It was strongly alkaline, and gave precipitates with the general alkaloidal reagents. From one to two milligrammes of this substance, injected into frogs, caused decreased activity of the heart, violent trembling, and death. The heart was found in diastole, and full of blood, and the brain slightly congested. However, the presence of this substance in the bodies of persons who have died of cholera does not prove that its production is due to the cholera bacillus.

POUCHET extracted from cholera stools, with chloroform, an oily base belonging to the pyridine series. It readily reduces ferric as well as gold and platinum salts, and forms an easily decomposable hydrochloride. It is a violent poison, irritating the stomach, and retarding the action of the heart. Subsequently, he obtained an apparently identical substance from cultures of KOCH'S comma bacillus.

In 1887, BRIEGER made a report of his studies on the chemistry of the cholera bacillus. He used pure cultures on beef-broth (fleischbrei), which was rendered alkaline by the addition of a 3 per cent. soda solution. These were kept at from 37° to 38°. After twenty-four hours, cadaverine was found to be present. Older cultures furnished very small quantities of putrescine, but cultures on blood-serum yielded much larger amounts of this base. While cadaverine and putrescine cannot be said to be poisonous, they do cause necrosis of tissue into which they are injected, and their formation by the cholera bacillus may account for the necrotic tissue in the intestine in the disease. The lecithin of the beef-broth was slowly acted upon by the germs, but with age the amount of choline increased, reaching its maximum during the fourth week.

Creatine proved still more resistant to the action of the germs; but, after six weeks, a considerable quantity of creatinine was isolated, and a smaller amount of methyl-guanidine. The latter is very poisonous, causing muscular tremors and dyspnœa. The presence of methyl-guanidine indicates that the comma bacillus acts as an oxidizing agent, since creatine yields methyl-guanidine only by oxidation.

BRIEGER succeeded in finding, in addition to the above-mentioned ptomaïnes, which are common products of putrefaction, two poisons which he considers as specific products of the comma bacillus. One of these, found in the mercuric chloride precipitate, is a diamine, resembling trimethylenediamine. It produced muscular tremor and heavy cramps. In the mercury filtrate was found another poison, which, in mice, produced a lethargic condition; the respiration and heart's action became slow, and the temperature sank, so that the animal felt cold. Sometimes there was bloody diarrhœa.

BRIEGER and FRÄNKEL found that the insoluble proteid which they obtained from cultures of the cholera bacillus, when suspended in water and injected subcutaneously in guinea-pigs, caused death after from two to three days. Section showed inflammatory swelling and redness of the subcutaneous tissue, extending into the muscles for some distance about the point of injection, but no necrosis. There was no change in the intestines and no effusion into the peritoneum. In some instances there were evidences of beginning fatty degeneration of the liver. Upon rabbits this substance, even in large doses, was without effect.

In endeavoring to obtain immunity in guinea-pigs against cholera, GAMALEIA employs cultures which have been sterilized at 120°. Subcutaneous injections of these cause transient œdema, and the animals soon recover. The high temperature destroys not only the bacillus, but renders inert certain "ferment-like" products. However, if the cultures be sterilized at 60°, large doses (10 c.c. per kilogramme, body weight) cause death, injected intravenously in rabbits, and a less amount produces marked symptoms. The animals refuse food, and a diarrhœa, which may continue for hours, appears. Often there is cloudiness of the cornea and retention of urine, which is albuminous. The animals recover very slowly. In this connection BOUCHARD remarks that in 1884 he obtained by the intravenous injection of the urine of a cholera patient in rabbits muscular tremor, cyanosis, albuminuria, and diarrhœa, but that he has never succeeded in inducing these symptoms with the cholera vibrio.

PETRI finds that the comma bacillus produces in solutions of peptone large amounts of tyrosin and leucin, a small quantity of indol, fatty acids, poisonous bases, and a poisonous proteid. The proteid resembles peptone in its behavior toward heat and chemical reagents, and is designated by PETRI as "toxopeptone." It is not precipitated by heat or concentrated nitric acid, nor by potassium ferrocyanide and acetic acid, nor by ammonium sulphate added to saturation. With sodium phospho-tungstate it gives a precipitate which clears up on the application of heat. The precipitate with tannic acid is insoluble in an excess of the precipitant. It gives the biuret reaction perfectly, but responds to Millon's test but feebly.

In quantities of 0.36 of a gramme per kilogramme and more it is fatal to guinea-pigs within eighteen hours. It produces muscular tremor and paralysis. Post-mortem shows an effusion into the peritoneal cavity, marked injection of the bloodvessels of the intestines, and isolated hemorrhagic spots.

This proteid is not rendered inert by a temperature of 100°. PETRI does not claim that he has obtained a chemically pure body, but supposes that it is contaminated with more or less unchanged peptone.

SCHOLL has studied the chemical products of the cholera bacillus when grown under anaërobic conditions. Fresh eggs were sterilized and inoculated in the usual way. The eggs, after being kept for eighteen days at 36°, were opened. The contents smelled intensely of hydrogen sulphide, but not of amines. The albumin was completely fluid, while the yolk was more solid and of a dark color.

Five c.c. of the fluid contents were injected into the abdomen of a guinea-pig. Soon the posterior extremities were paralyzed, and after ten minutes the paralysis became general, the animal lying on the side. After five minutes more convulsive movements of the extremities began, and forty minutes after the injection the animal was dead. Section showed the vessels of the small intestines and stomach highly injected, a colorless effusion in the peritoneal cavity, and the heart in diastole.

The albuminous content of the egg was poured into ten times its volume of absolute alcohol. The precipitate was collected and washed with alcohol until a colorless filtrate was obtained. The precipitate was then digested for fifteen minutes with 200 c.c. of water and filtered. Eight c.c. of the filtrate was injected into the abdomen of a guinea-pig. Paralysis resulted immediately, and within one and one-fourth minutes the animal was dead. Section showed marked injection of the vessels of the small intestines, a bloody transudate in the peritoneal cavity and the heart in diastole.

The poisonous proteid was rendered inert by a temperature of 100°; it was not altered by short exposure to 75°, but attempts to evaporate the solution at 40° in vacuo over calcium chloride destroyed the poisonous properties. The proteid was finally precipitated from its aqueous solution by a mixture of alcohol and ether. It was washed with ether and the ether allowed to evaporate spontaneously. A small bit of this proteid proved fatal to guinea-pigs, and the same post-mortem changes were found as given above. SCHOLL classes this proteid among the peptones. It is not precipitated by heat or concentrated nitric acid, singly or combined, nor by ammonium sulphate. It gives the xanthoproteid and biuret reactions. SCHOLL regards this as the true poison of cholera, and points out its difference from the proteid of BRIEGER and FRÄNKEL and that of PETRI.

BUJWID found that on the addition of from five to ten per cent. of hydrochloric acid to bouillon cultures of the cholera bacillus there was developed after a few minutes a rose-violet coloration which increased during the next half hour and in a bright light showed a brownish shade. The coloration is more marked if the culture is kept at about

37°. In impure cultures this reaction does not occur. The **Finkler-Prior** bacillus cultures give after a longer time a similar, but more of a brownish coloration. Cultures of many other bacilli were tried and failed to give this reaction.[1]

Brieger found that this color is due to an indol derivative. In cholera cultures on albumins he obtained indol by distillation with acetic acid.

Bujwid has made a further contribution to our knowledge of the "cholera-reaction." His conclusions are as follows:

(1) Five to ten per cent. of hydrochloric acid added to cholera cultures produce a rose-violet coloration, which is characteristic of the comma bacillus.

(2) No other bacterium gives the same coloration under the same conditions.

(3) The coloration appears in such cultures which are from ten to twelve hours old, so that this test can be used for diagnostic purposes, and will give results before they can be obtained by plate cultures.

(4) Impure cultures do not give this reaction.

Dunham finds the best medium for the "cholera-reaction" to be a one per cent. alkaline peptone solution with one-half per cent. of common salt. **Bujwid** prefers a two per cent. feebly alkaline peptone solution with salt. **Jadassohn** finds that gelatin cultures give the reaction both before and after the liquefaction of the gelatin. The undissolved gelatin, after the addition of hydrochloric or sulphuric acid, becomes rose-violet.

Cohen claims that cultures of other bacilli give a similar coloration, but **Bujwid** explains that the results obtained by **Cohen** were due to the use of impure acids, which contained nitrous acid. **Salkowski** agrees with **Bujwid**, and states that, when acids wholly free from nitrous acid are used, the reaction is characteristic of the comma bacillus. He explains the reaction by supposing that the germ produces nitrous acid, which exists in the culture as a nitrite. On the addition of an acid the nitrous acid is set free, and acting upon the indol, which is also present, gives the coloration.

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[1] oehl deserves the credit of being the first to call attention to this n, though his work was evidently unknown to Bujwid at the time e latter published his report.

From a very exhaustive research on the importance of this test PETRI comes to the following conclusions:

(1) Seven pure cultures of the cholera germ from as many sources gave the reaction with equal distinctness.

(2) Of one hundred other bacteria tested in the same way twenty gave a red coloration. In nineteen of these the coloration is due to the nitroso-indol reaction of Baeyer. The twentieth (anthrax) gave a color which is not due to indol.

(3) In case of the cholera germ and the others as well, the reaction is due to the reducing action of the bacteria on nitrates. The reaction is most marked at blood-temperature and with the cholera bacillus; it is least distinct with the bacilli of FINKLER and MILLER.

(4) None of these bacteria convert ammonia into nitrite.

(5) The simple addition of sulphuric acid is sufficient to give the test, which, however, is most marked when the nutritive solution contains 0.01 per cent. of nitrate.

(6) The reaction is most marked if the sulphuric acid be added after the addition of a very dilute nitrite solution.

SCHUCHARDT calls attention to the fact that VIRCHOW observed a red coloration on the addition of nitric acid to filtered cholera stools in 1846. GRIESINGER, in 1885, also made mention of the production of a red coloration in rice-water stools on the addition of nitric acid.

A "cholera-blue" has also been observed by BRIEGER in cultures in meat extract containing peptone and gelatin. This substance, which is yellow by reflected, and blue by transmitted light, is developed by the addition of concentrated sulphuric acid to the culture. It may be separated from the "cholera-red" as follows: Treat the culture with sulphuric acid, then render alkaline with sodium hydrate, and extract with ether. Evaporate the ether, and remove the "cholera-red" with benzol, then again dissolve the "cholera-blue" in ether. The characteristic absorption bands for this coloring matter begin in the first third of the spectrum, between E and F, and darken all of the zone lying beyond.

WINTER and LESAGE treat a bouillon culture of the cholera germ with sulphuric acid, dissolve the precipitate in an alkaline medium, reprecipitate with acid, and redissolve in ether, which on evaporation leaves oily drops, which, on cooling, form a yellow mass of the appearance of a fat This substance is insoluble in water and acids, soluble in alkalies and ether. It melts at 50°, and does not lose its virulence on being boiled with alcohol rendered feebly alkaline. The virulence of a culture and the amount of this substance contained therein are in direct proportion to each other.

Small doses of this substance (1 milligramme to 100 grammes of body weight of the animal) in feebly alkaline solution introduced into the stomachs of guinea-pigs cause, as a rule, within from four to six hours, a chill, and death after twenty-four hours. With larger doses the temperature falls after from one-half to one hour, and death results within from twelve to twenty hours. Smaller doses cause a less marked reaction and the animal recovers within twenty-four hours. If killed within this time the animal shows a choleraic condition. Rabbits succumb only after repeated subcutaneous injections. The substance can be extracted from the muscles, liver, kidneys, and urine of the poisoned animals. It can also be obtained from cultures of a cholera infantum germ. The fact that this poison belongs neither to the ptomaïnes nor albumins is of interest.

CUNNINGHAM describes ten species of the common bacillus, one of which does not liquefy gelatin, and fails to respond to the cholera reaction. He also states that there are cases of undoubted cholera in Calcutta in which the common bacillus is wholly wanting.

TETANUS.—In 1884, NICOLAIER, by inoculating 140 animals with earth taken from different places, produced symptoms of tetanus in 69 of them. In the pus which formed at the point of inoculation he found micrococci and bacilli. Among the latter was one which was somewhat longer and slightly thicker than the bacillus of mouse-septicæmia. In the subcutaneous cellular tissue he found this bacillus alone, but could not detect it in the blood, muscles, or nerves. Heating the soil for an hour rendered the inoculations with it harmless. In cultures, NICOLAIER was unable to separate this bacillus from other germs, but inoculations with mixed cultures produced tetanus. In the same year, CARLE and RATONE induced tetanus in lower animals by inoculations with matter taken from a pustule on a man just dead from tetanus. In 1886, ROSENBACH made successful inoculations on animals with matter taken from a man who had died from tetanus consequent upon gangrene from frozen feet. With bits of skin taken from near the line of demarcation, he inoculated two guinea-pigs on the thigh; tetanic symptoms set in within twelve hours, and one animal died within eighteen, and the other within twenty-four hours. The symptoms corresponded exactly with those observed in the "earth tetanus" of NICOLAIER, and the same bacillus was found. With mixed cultures of this, ROSENBACH was also able to cause death by tetanus in animals. BEUMER had under observation a man who died from lockjaw following the sticking of a splinter of wood under his finger-nail. Inoculations of mice and rabbits with some of the dirt found on the wood led to tetanus. The same observer saw a boy die from this disease following an injury to the foot from a sharp piece of stone. White mice inoculated with matter from the wound, and those inoculated with dirt taken from the boy's play-ground, died of tetanus. The bacillus of NICOLAIER was again detected. GIORDANO reports the case of a man who fell and sustained a complicated fracture of the arm. He remained on the ground for some hours, and when assistance came the muscles and skin were found torn and the wounds filled with dirt. On the fifth day he showed symptoms of tetanus, from which he died on the eighth day. Inoculations and examinations for the bacillus were again successful. FERRARI also made successful inoculations with the blood taken during life from a woman with tetanus after an ovariotomy. **Hocksinger** has confirmed the above-mentioned observations by carefully conducted experiments, the material for which was furnished by a case of tetanus arising from a very slight injury to the hand, the wound being filled with dirt. **Shakespeare** has succeeded in inducing tetanus in rabbits by inoculating them with matter taken from the medulla of a horse and of a mule, both of which had died from traumatic tetanus. These uniform observations leave no room to doubt that tetanus is often, at least, due to a germ which exists in many places in the soil, and that the disease is transmissible by inoculation.

Bonome observed nine cases of tetanus among seventy persons injured by the falling of a church from the earthquake at Bajardo. The bacillus of **Nicolaier** was detected in the wounds, and animals inoculated with the lime-dust of the fallen building died of tetanus. Of many persons injured by the falling of another church at the same time, none had tetanus, and animals inoculated with the lime from this church suffered no inconvenience.

The same experimenter found the bacillus in the wound of a sheep which died from tetanus after castration.

Beumer found the tetanus bacillus in the sloughing tissue of the umbilical cord of a child which was taken ill on the sixth day after birth, and died four days later from tetanus. From this he concludes that tetanus neonatorum and "earth tetanus" are identical, and advises that the cord should be dressed antiseptically.

Kitasato has succeeded in isolating the bacillus of Nicolaier by growing the mixed cultures, from the pus of a wound on a man who died from tetanus, at a high temperature (80°), and subsequently developing the germ under hydrogen. The bacillus grows only in the absence of air, and not in carbonic acid. It develops on agar, blood-serum, and gelatin, the last of which it gradually liquefies with the formation of gas. The growth is more vigorous when the nutritive medium contains from 1.5 to 2 per cent of grape-sugar.

In 1888 **Belfanti** and **Pescarolo** found in the pus of a wound, which was followed by tetanus, a bacillus which they believed to differ morphologically from that of Nicolaier and Rosenbach, and which in pure cultures induces tetanus in animals. The number of animals experimented upon was great and included mice, guinea-pigs, frogs, rabbits, pigeons, geese, sparrows, a chicken, and a dog. The pigeons, chicken, geese, and frogs proved immune. After subcutaneous injections a bloody œdema appeared at the place of inoculation and pus formed in small quantity. Paralysis first appeared and was followed by convulsions and opisthotonos. Later studies lead BELFANTI and PESCAROLO to conclude that their bacillus is really that of NICOLAIER, but differing somewhat from that of KITASATO. KITASATO states positively that the germ which he has isolated is absolutely anaërobic, while the Italians find that theirs will not only grow aërobically, but when so grown will induce a classical tetanus.

LAMPIASI found in the blood from various organs of a man who died from so-called spontaneous tetanus, and in two cases of tetanus in mules, a spore-forming bacillus, which in pure cultures induced tetanus in animals. This bacillus is wholly different morphologically from that of Nicolaier.

WIDENMANN reports a very interesting case of a boy who fell from a wall and wounded his face on a piece of vine-stake in the earth. The boy died of tetanus, and the splinters extracted from the face and the earth about the stake were examined. The splinter was introduced under the skin of a mouse, which died thirty hours later of tetanus. In the pus formed about the splinter numerous microörganisms, among which a micrococcus and a short, thick bacillus abounded, were found, but in none of the many animals experimented upon could the bacillus of NICOLAIER be detected. In animals inoculated with the earth, however, the NICOLAIER germ was found. WIDENMANN concludes that the so-called tetanus bacillus is found in most cases on account of its very wide distribution in the soil and not as a result of its causal relation to the disease.

FLÜGGE has produced tetanus in animals without being able to find the bacillus of NICOLAIER, and WYSSOKOWITSCH has examined an earth which did not induce tetanus, but which caused suppuration, and in the pus the NICOLAIER bacillus was found to be abundant. With the pus obtained from three cases of tetanus neonatorum due to omphalitis KISCHENSKY induced tetanus in animals. The pus contained pyogenetic micrococci and a short bacillus, but the germ of NICOLAIER could not be detected.

Although KITT claims that his tetanus bacillus is identical with that of KITASATO (which is now regarded as a pure culture of the germ of NICOLAIER), the former liquefies solid blood-serum and the latter does not. Bacteriologists generally agree that the NICOLAIER bacillus is found only at the place of inoculation and that it is never present in the blood or internal organs, yet SHAKESPEARE, as we have seen, induced tetanus in rabbits by inoculating them with matter taken from the medulla of a horse and that of a mule, both of which had died of tetanus. The bacillus which has been so well studied by TIZZONI and CATTANI has certain constant biological differences from that of KITASATO.

PLA has studied eight cases of traumatic tetanus both by cultures and by inoculation of animals. In none has he found the germ of NICOLAIER. Moreover, since tetanus was induced in animals by bits of matter taken from the spinal cord, the NICOLAIER germ could not have been the cause, if, as bacteriologists now teach, this germ is never found save at the place of inoculation.

BRIEGER has obtained in the mixed cultures of the germ of NICOLAIER and ROSENBACH four poisonous substances. The first, tetanine, which rapidly decomposes in acid solutions, but is stable in alkaline solutions, produces tetanus in mice when injected in quantities of only a few milligrammes. The second, tetanotoxine, produces first tremor, then paralysis followed by severe convulsions. The third, to which no name has been given, causes tetanus accompanied by free flow of the saliva and tears. The fourth, spasmotoxine, induces heavy clonic and tonic convulsions.

BRIEGER has also isolated tetanine from the amputated arm of a man with tetanus, thus showing that this chemical poison is formed in the body as well as in the artificial cultures.

BRIEGER and FRÄNKEL obtained a "toxalbumin" from a culture of KITASATO's germ in bouillon containing grape-sugar. This substance is soluble in water, and when injected in small amounts subcutaneously in guinea-pigs, tetanus appears in about four days, and soon terminates fatally. On the other hand, cultures of the bacillus of TIZZONI and CATTANI in bouillon with sugar fail to produce any chemical poison, but the cultures in gelatin are highly poisonous after filtration through porcelain. Even one-half cubic centimetre of the latter induces the disease and death in rabbits weighing from one and a half to two kilogrammes. Death results never later than three days, while, as has been seen above, the first symptoms induced by the poison from the bacillus of KITASATO usually appear on the fourth day. BRIEGER and FRÄNKEL obtained their proteid by precipitation with absolute alcohol, but the addition of this agent to cultures of the germ of TIZZONI and CATTANI destroys its poisonous properties. The active substance of the Italian germ was obtained either (1) by dialysis, solution in water, and evaporation in a vacuum; or (2) by precipitation with ammonium sulphate, separation by dialysis, and drying in a vacuum. This poisonous body is soluble in water, non-dialyzable, destructible by a temperature above 60°, and by treatment with concentrated mineral acids, and is unaffected by alkalies or by prolonged treatment with carbonic acid gas. It contains a ferment which liquefies gelatin and digests fibrin. This peptonizing ferment is active only in alkaline solution, and is present in the bouillon cultures which are not poisonous; therefore, the poison and the peptonizing ferment must be two distinct bodies. However, on account of the properties which we have mentioned, TIZZONI and CATTANI conclude that the poison also belongs to the soluble ferments or enzymes.

BUSCHETTINI has studied the distribution of this poison through the body and its elimination in the following manner:

Animals were poisoned by injections of the substance prepared by TIZZONI and CATTANI, and just before death they were killed and bits of various organs rubbed up with sterilized water were injected into other animals. Emulsions from the liver and supra-renal capsules were invariably without effect, while those from the kidney were constantly poisonous. This is supposed to prove that the poison is eliminated by the kidney. The blood taken from the vena cava was found to be poisonous in three out of four experiments. When the injections were made under the skin the lumbar cord was active in four out of eight cases, and in all, when the injections were made directly into the sciatic nerve. On the other hand, when the inoculations were made under the dura mater, the brain was found to be active while the lumbar cord remained inactive. From these experiments it is concluded that the poison not only circulates in the blood, but is deposited in the central nervous system.

A. BABES prepared, from cultures made by V. BABES and PUSCARIA in agar containing no peptone, an albumose which causes tetanus in animals.

FABER finds in a mixed culture a poisonous proteid body which resembles closely, so far as it has been studied, that of TIZZONI and CATTANI. FABER lays much stress upon the arguments in favor of this substance being a soluble ferment. With this proteid, convulsive movements first appear and become very distinct in the muscles about the point of injection. In case very small amounts are employed, the convulsive movements do not become general and the animal finally recovers.

PEYRAUD claims to have secured immunity in animals against "earth tetanus" by giving to them strychnia in gradually increased doses. NOCARD could not confirm this claim.

According to LEDANTES, the poisonous arrows of the natives of the New Hebrides are prepared as follows: The points, which are usually made from human bones, are first covered with a vegetable resin, then smeared with the slime of swampy places.

LIERMANN found that material taken from the arm of a man who had died from tetanus, and who had been buried for two and one-half years, induced tetanus in animals. This would seem to show that the poison retains its virulence for a long time. In this material there were found nine kinds of bacteria, but none of these in pure culture, or in mixed culture, induced the disease. This is explained by the supposition that non-pathogenic bacteria may receive toxicogenic properties from the media in which they grow.

TUBERCULOSIS.—Whatever may be the ultimate verdict concerning the curative properties of KOCH's tuberculin, its employment has made us familiar with the action of the chemical products of the bacillus tuberculosis on man. Unfortunately, KOCH has given us but little information concerning the nature of his tuberculin, and the little which he has given us has been to some extent misleading. We would not imply that he has intentionally been misleading. Indeed, we believe that such was not his intention. He speaks of the agent as an extract of a pure culture of the bacillus tuberculosis with 50 per cent. glycerin. One would infer from KOCH's statements that tuberculin is prepared by extracting the bacterial cells with 50 per cent. glycerin, and that the bacterial products are not present. But, as has been shown by HUEPPE and SCHOLL, the proteids of the cells of the bacillus tuberculosis cannot be extracted with 50 per cent. glycerin. Moreover, the same investigators have prepared a fluid identical in physical properties, in chemical reactions, and in its effects on animals, with KOCH's fluid, by each of the three following methods:

1. Cultures of the bacillus are filtered, sterilized by heat, and concentrated.

2. The supernatant, fluid portion of the culture is decanted from the mass of germs at the bottom of the flask, and then concentrated.

3. The culture is freed from germs by filtration through a Chamberland filter, and concentrated.

These fluids contain: 1, the constituents of the nutritive medium which have not been altered by the growth of the germ, such as glycerin, albumins, albumoses, and peptones; 2, the bacterial products, which may possibly belong to the ptomaïnes, the bacterial albumins or albumoses and bacterial ferments; and 3, any constituents of dead, broken-down bacilli which may have passed into solution. To which of these constituents the action of the fluid is due has not been positively determined. However, from the similarity in the action of this fluid with that of the bacterial products of other germs, we seem justified in assuming that these constitute the active principle.

As early as 1888, **HAMMERSCHLAG** found a poisonous proteid among the products of the growth of this germ. More recently he finds that as much as 27 per cent. of the cellular substance of the bacillus tuberculosis is soluble in alcohol and ether. In this extract there is, in addition to fat and lecithin, a poison which induces in rabbits and guinea-pigs convulsions followed by death. The part insoluble in alcohol and ether consists of cellulose and proteids. **HAMMERSCHLAG** has also prepared from cultures of this bacillus a "toxalbumin" which, when injected subcutaneously in rabbits, causes an elevation of temperature of from 1° to 2°, which continues for a day or longer.

ZUELZER has reported the isolation of a poisonous ptomaïne from agar cultures of the bacillus tuberculosis. He says that the injection of 1 centigramme or less of this substance subcutaneously in rabbits or guinea-pigs causes, after from three to five minutes, increased frequency of respiration (to 180 per minute?) and an elevation of temperature of from 0.5° to 1°. He also reports marked protrusio bulbi as a constant symptom; the eyes become very bright and the pupils are dilated. From two to three centigrammes suffice to kill rabbits, death occurring in from two to four days. The place of injection is reddened, and hemorrhagic spots are formed in the mucous membrane of the stomach and small intestines. In two instances from 15 to 20 cubic centimetres of clear fluid were found in the peritoneal cavity.

Baumgarten draws the following conclusions from his experiments with tuberculin on rabbits with inoculation tuberculosis:

It causes an exudative inflammation in the vascular tissue about the tubercle, and in this way the tuberculous tissue may be isolated and, when situated superficially, removed. In some cases, however, after the prolonged employment of the agent, the tuberculous tissue itself may, under the influence of the exudative fluid and the polynuclear leucocytes, break down and form abscesses. The bacilli themselves are in no way harmed by the use of tuberculin, and, after its constant employment for months, they retain their original form and lose none of their virulence. Some preparations seem to show that the bacilli multiply more rapidly when the injections are made, but a positive statement on this point is reserved until further studies have been made. It is certain, however, that the non-tubercular tissue of animals acquires no immunity against the disease from the injections. This is shown by the appearance of metastatic foci in animals in which from seven to twelve grammes of the original lymph (an amount which would be equivalent to from seventy to one hundred and eighty grammes in man) has been injected. It is further shown by the fact that in some animals treated subcutaneously, tubercles have appeared at the point of injection.

Prudden and **Hodenpyl** summarize the results which they have obtained by the inoculation of animals with dead tubercle bacilli as follows: "These dead tubercle bacilli are markedly chemotactic. When introduced in considerable amount into the subcutaneous tissue or into the pleural or abdominal cavities, they are distinctly pyogenetic, causing aseptic localized suppuration. Under these conditions they are capable, moreover, of stimulating the tissues about the suppurative foci to the development of a new tissue, closely resembling the diffuse tubercle tissue induced by the living germ. We have found that dead tubercle bacilli introduced in small numbers into the bloodvessels of the rabbit largely disappear within a few hours or days, but that scattering individuals and clusters may remain here and there in the lungs and liver, clinging to the vessel walls for many days without inducing any marked changes in the latter. After a time, however—earliest in the lung, later, as a rule, in the liver—a cell proliferation occurs in the vicinity of these dead germs, which leads to the formation of new multiple nodular structures bearing a striking morphological resemblance to miliary tubercles. There is in them, however, no tendency to cheesy degeneration and no evidence of proliferation of the bacilli, but rather a steady diminution in their number. It seems to us that the new structures originate in a proliferation of the vascular endothelium under the stimulus of the dead and disintegrating germs."

MAFFUCCI finds that cultures of the tubercle bacillus (from a mammal), when grown from one to six months on glycerin, blood-serum, or liquid blood-serum, and then sterilized by being repeatedly heated to from 65° to 70°, produces in guinea-pigs, when employed subcutaneously, a progressive marasmus, which terminates fatally within from fourteen days to five or six months. He also finds that eggs inoculated with sterilized cultures of the chicken tuberculosis bacillus produce chickens which are feeble and soon die of emaciation. In neither the guinea-pigs nor chickens could he find any tubercles. This author, unfortunately, does not state positively whether the bacilli employed in his experiments on guinea-pigs were obtained from man or some other mammal.

CROOKSHANK and HERROUN report the isolation of a ptomaïne and an albumose not only from artificial cultures of the bacillus, but also from bovine tuberculous tissue. The ptomaïne is reported as causing an elevation of temperature in tuberculous, and a depression in healthy, animals. "The albumose, whether obtained from pure cultivations of the bacillus, or from tuberculous tissue, produced a marked rise of temperature in tuberculous guinea-pigs. On the other hand, in an experiment tried on a healthy guinea-pig, there was an equally well-marked fall of temperature."

DIPHTHERIA.—That the **LÖFFLER** bacillus is *a* cause of diphtheria no one can now deny. The fact that this germ, although found only at the seat of inoculation, causes marked systemic disturbances, indicates that its action must be due to its soluble products. This was early recognized by **LÖFFLER**, who in 1887 attempted to ascertain the nature of the poison. A flask of bouillon containing peptone and grape-sugar was, three days after it had been inoculated with the bacillus, evaporated to 10 c.c., and this was injected into an animal, but was without effect. A second flask of the same material was extracted with ether, but this extract was also found to be inert. Next, some neutral beef broth was extracted with glycerin some four or five days after it had been inoculated with the bacillus. The glycerin extract, when treated with five times its volume of absolute alcohol, deposited a voluminous, flocculent precipitate, which was collected, washed with alcohol, dried, and dissolved in a little water. A further precipitation with alcohol and a current of carbonic acid gas secured a white substance, and the injection of from 0.1 to 0.2 gramme of this, dissolved in water, subcutaneously in guinea-pigs, caused marked pain followed by a fibrous swelling with hemorrhage into the muscles and œdema, terminating in necrosis. From these studies **LÖFFLER** concluded that the poison belongs to the enzymes.

ROUX and **YERSIN** found that bouillon cultures from which the bacillus had been removed by filtration through a Chamberland filter are poisonous, especially cultures which are four or five weeks old. The results obtained varied with the amount of the fluid, the species of animal, and the method of administration. The effects observed were a serous exudation into the pleural cavity, a marked, acute inflammation of the kidney, fatty degeneration of the liver, especially after injection into a bloodvessel, and œdematous swelling in the surrounding tissue after subcutaneous inoculation. In some instances, in dogs, rabbits, and guinea-pigs, paralysis, generally in the posterior extremities, followed. The action of the poison was found to be very slow, and, as a rule, death occurred days, and in some instances weeks, after the inoculation, and was preceded by marked emaciation.

The cultures first employed were seven days old; older cultures (six weeks) contain more of the poison, and the symptoms appear within a few hours. In cultures especially rich in the poison, a small amount (from 0.2 to 2 c.c.) injected under the skin in guinea-pigs suffices to induce the symptoms. Mice and rats are markedly insusceptible, but succumb to large doses.

Heating to 100° for twenty minutes renders the poison inert, and a temperature of 58° maintained for two hours markedly lessens its virulence.

The poisonous substance is precipitated by absolute alcohol, and is carried down mechanically on the addition of calcium chloride to the filtered cultures. These investigators agree with LÖFFLER that the poison belongs to the enzymes. The great toxicity of this substance is indicated by the statement of ROUX and YERSIN that 0.4 milligramme suffices to kill eight guinea-pigs or two rabbits, and that 2 centigrammes of the calcium chloride precipitate, containing about 0.2 milligramme of the pure poison, will kill a guinea-pig within four days.

BRIEGER and FRÄNKEL have made a very complete study of the chemical products of the LÖFFLER bacillus. They employed cultures of bouillon and peptone containing from five to six per cent. of glycerin, and others containing ten per cent. of sterile, fluid blood-serum. The latter were found to be most suitable. In these the bacilli grow most abundantly. In all cases they confirmed the statement of ROUX and YERSIN that the cultures, at first alkaline, become strongly acid, and finally again alkaline, with the exception that the glycerin cultures remained acid.

For the removal of the bacteria two methods were employed. First the bacilli were destroyed by heat. When a temperature of 100° was employed the cultures were rendered inert, but it was found that exposure for from three to four hours to a temperature of 50° was sufficient to destroy the germs, while the virulence of the chemical products was not affected. The second method of removing the bacteria consisted of filtration through a Chamberland filter. The germ-free filtrate could be heated to 50° without loss of toxicity, while a temperature of 60° rendered it inert. In the majority of the experiments the filtration method was used and in this way a large quantity of a poisonous fluid of uniform strength was obtained.

Varying amounts of this fluid were used upon animals, mostly guinea-pigs and rabbits, and it was found that the effects varied with the quantities employed and the methods of administration. The symptoms appeared most promptly when the injections were made directly into a bloodvessel. Of four rabbits which were given subcutaneously respectively 1, 2½, 5, and 10 c.c. of the filtrate on December the 28th, the first died January 4th; the second, January 2d; the third, December 31st; and the fourth, December 30th. In all cases in which death did not occur too early, paralysis appeared. The limbs were first paralyzed, and this was true whether the fluid was administered intravenously or subcutaneously. The post-mortem appearances were identical with those observed after inoculation with the bacillus, with the exception of the absence of the pseudo-membrane. After subcutaneous injection there was a gelatinous, grayish-white, sometimes reddish, œdematous fluid formed at the point of injection; and, after larger doses, necrosis. In cases in which death was delayed, there were effusions in the pleura, fatty degeneration of the liver, and inflammation of the kidneys. Especially marked were these cellular changes in rabbits which were treated with small amounts intravenously.

BRIEGER and FRÄNKEL conclude this part of their report with the following statement: "We have shown that the LÖFFLER diphtheria bacillus produces in its cultures a poisonous, soluble substance, separable from the bacteria, which causes in susceptible animals the same phenomena which are induced by inoculation with the living microörganism. We have further shown that this substance is destroyed by a temperature over 60°, but that it can be heated to 50°, even in the presence of an excess of hydrochloric acid, without being destroyed. This last fact is contrary to the assumption that the chemical poison of the diphtheria bacillus is a ferment or enzyme."

The fluid was tested for basic products, but with wholly negative results, except that small amounts of kreatinin and cholin were found. It was also distilled at from 20° to 35° in a vacuum, and the distillate was found to be inert.

The poisonous substance was found to be insoluble in alcohol, soluble in water, and non-dialyzable. It was precipitated by saturation with ammonium sulphate.

The substance was obtained by allowing the germ-free filtrate, after being rendered feebly acid with acetic acid, to fall into a large volume of absolute alcohol. It was purified by repeated solution in water and precipitation with alcohol. It contains a large amount of sulphur, and responds to the biuret and Millon tests. It is, therefore, classified among the albumins. Since it is not precipitated by saturation with magnesium sulphate at 30°, it cannot belong to the globulins. The fact that it is precipitated by saturation with ammonium sulphate, and that it does not dialyze, shows that it is not peptone. It is, therefore, classified by BRIEGER and FRÄNKEL among the albumins, and is designated as a "toxalbumin."

The special reactions and the results of an ultimate analysis of this substance have already been given (page 20).

This proteid induces in animals all the symptoms and post-mortem appearances which have been mentioned as following the administration of the filtered cultures. It is to be noted that the injection of small quantities of this proteid (2½ milligrammes per 1 kilogramme of the body-weight of the animal) does not produce its effects until after the lapse of weeks, and possibly months. This peculiarity in action distinguishes this class of substances from all other chemical poisons, and it has received as yet no satisfactory explanation. There is no reason for believing that the body obtained by BRIEGER and FRÄNKEL is chemically pure, and until it has been obtained in this condition we can only speculate concerning its true nature.

It should be remarked that the LÖFFLER bacillus shows not only marked morphological variations, but that it is very variable in its virulence, some cultures having been obtained which are wholly without effect upon animals. From cultures of this kind **Brieger** and **Fränkel** prepared a non-poisonous albumin differing in its ultimate composition and in many of its chemical reactions from the poisonous one.

Fränkel has been unable to secure immunity in animals against diphtheria by the employment of small doses of the "toxalbumin." If the dose is large enough the animal dies. If it is smaller, the animal seems to become more susceptible and succumbs more readily to inoculations with the germ. While this is true of the filtered culture, it is not the case with that which has been sterilized by heat. **Fränkel** finds that if from 10 to 20 c.c. of a culture of the bacillus three weeks old, which has been heated for one hour at from 65° to 70°, be injected under the skin of the abdomen of guinea-pigs, immunity against subsequent inoculation with the virulent germ is secured, provided that the inoculation is not made earlier than the fourteenth day after the treatment with the sterilized culture. He thinks that the culture contains two specific albumins, one of which is poisonous, while the other gives immunity. The former is destroyed by a temperature of from 65° to 70°, while the other retains its characteristic properties. He admits the possibility that the poisonous albumin may be converted into the other form by the high temperature. He finds that the modified culture, which gives immunity, is of no service for therapeutic purposes, and that if an animal be treated with it directly after inoculation with the germ, death is not retarded, but is hastened. From these experiments he concludes that the vaccination albumin at first lessens, and subsequently increases the resistance of the animal.

Sprouck and his students have confirmed the above statements concerning the toxicity of the germ-free cultures of this bacillus. They have also called attention to the albuminuria following the employment of this poison. In the urine they find casts, white, and sometimes red, blood-corpuscles. Microscopic examination of the kidney after death shows the same changes which are observed in the diphtheritic nephritis of children. BABES also finds that the germ-free cultures produce the parenchymatous degenerations of the internal organs which are found in the human body.

TANGL has shown that the chemical poison is formed in the body as well as in culture-flasks. A large piece of pseudo-membrane was macerated in water in an ice-chest for twenty-four hours, and then filtered through porcelain. The filtrate, injected into animals, produced all the symptoms which have been obtained by a similar employment of artificial cultures. TANGL also observed that in some cases in which the animals were inoculated with the sterilized culture through the mucous membrane a pseudo-membrane formed at the point of injection.

SUPPURATION.—As early as 1879, LEBER concluded from his observation on infective keratitis that the aspergillus must produce certain soluble products which diffuse through the cornea and set up an inflammatory action in the adjacent vascular tissue. In 1882, he showed that suppuration could be induced by the introduction of sterilized mercury and copper, and that the pus formed is free from germs. In 1884, he induced suppuration by the injection of cultures of the staphylococcus pyogenes aureus which had been sterilized by being boiled for hours. In 1888, the same investigator reported that he had found an alcoholic extract of the dried staphylococcus to be highly pyogenetic. From this extract he has prepared a crystalline body which he calls phlogosin. This substance is readily soluble in alcohol and ether, sparingly soluble in water, and it crystallizes in needles. The crystals can be sublimed, leaving no residue, and the sublimate, which forms in rosettes, still possesses the pyogenetic properties. Alkalies precipitate this substance from its solution in amorphous granules, which dissolve in acids, forming crystalline salts. LEBER refers to the observation of the botanist PFEFFER, who found that vegetable cells are attracted by certain chemical substances, and adopts the term chemotactic action (chemotactische Wirkung) to indicate the property of certain chemical agents of attracting leucocytes.

As has been stated, **Buchner** has found that the cells of many bacteria contain pyogenetic proteids. The amount of these substances in the cells varies with the kind of germ, and some species (the bacillus prodigiosus, for instance) seem to contain no such bodies. The bacillus pyocyaneus contains a large quantity of the proteid, and is suitable for lecture demonstration. The germs are taken from potato cultures and rubbed up with water. Then they are treated with about fifty volumes of a 0.5 per cent. solution of caustic potash. This forms in the cold a mucilaginous mass which dissolves at the temperature of the water-bath. After being heated for some hours the fluid is filtered through a number of small filters; the first portions should be refiltered. The filtrate is a greenish fluid (pyocyanin) which by the careful addition of acetic or hydrochloric acid (an excess is to be avoided) forms a voluminous precipitate (pyocyaneus proteid). This precipitate should be collected on a filter, washed with water, then suspended in water and a few drops of a soda solution added, when a dark-brown fluid, with a tendency to gelatinize in the cold, containing about 10 per cent. of the proteid, is obtained.

13.254 grammes of the moist bacteria yield 1.44 gramme of dry bacterial substance, and this after the treatment given above furnishes 0.2739 gramme of dry proteid = 19.3 per cent. This proteid leaves 11.52 per cent. of ash, which contains phosphoric acid, but consists principally of sodium chloride.

Much smaller amounts of proteid were obtained from other germs, but the Eberth germ, bacillus subtilis, lactic acid bacillus, red bacillus from potato, and staphylococcus pyogenes aureus furnished considerable quantities.

The chemotactic properties of these proteids were tested in the following manner: The dissolved proteid was placed in a spindle-shaped glass tube, and the tubes, sterilized by prolonged boiling, were introduced under the skin on the backs of rabbits with antiseptic precautions, and the ends of the tubes broken off subcutaneously.

After from two to three days the tubes were removed and found to contain, in addition to some of the proteid, several millimetres of fibrinous pus, which was examined microscopically and by the preparations of cultures, which invariably remained sterile. The proteid of the EBERTH bacillus was found to have specially marked pyogenetic properties.

Similar experiments were made with the following crystalline substances: the butyrate and valerianate of ammonia (each 1 per cent. solution), trimethylamin (2 per cent.), ammonia (2 per cent.), leucin, tyrosin and glycocol (1 per cent.), urea (5 per cent.), and urate of ammonia and skatol (1 per cent.). Glycocol and leucin only were found to have the chemotactic action, and with these this action was but slight compared with that of the bacterial proteids.

The next experiments were made with the object of ascertaining whether or not proteids similar to those derived from the bacteria would cause a like effect. The bacterial cellular proteids resemble very closely vegetable casein some of which was prepared from wheat gluten and tested as above. This proteid was found to be possessed of marked chemotactic properties. The subcutaneous injection of sterilized preparations of wheat-flour and ground peas were also found to cause suppuration. Negative results were obtained with starch and solutions of disodium hydric phosphate. From this it is concluded that the active agent in the flour is its casein.

Peptone was employed without effect, while gelatin was found to act energetically. Alkaline albuminates were prepared from muscle, liver, lungs, and kidney by treating finely divided portions of these organs with potash and proceeding as in the preparation of the bacterial proteids. All of these caused the formation of pus, and the preparations from the liver were found to be specially potent.

Similar preparations from blood and egg-yolk were active, while those from fibrin and the white of egg had no effect. Hemi-albumose was also found to be active, and this fact is placed in contrast with the negative result obtained with peptone.

One of the most interesting results was obtained by the daily injection of a chemotactic proteid directly into the blood. Before the first injection the proportion of white to red corpuscles was 1 : 318; on the second day, 1 : 126; on the third, 1 : 102; on the morning of the fourth, 1 : 73; on the afternoon of the fourth, 1 : 38. After this there was no further increase. The absolute number of red corpuscles remained unchanged, while the absolute number of the white multiplied sevenfold. The white corpuscles were on the first days often found in groups of from two to four, and later, of from ten to twenty. This seems to demonstrate that these substances cause an increased production of leucocytes. General leucocytosis was induced by the similar employment of vegetable casein and an alkaline albuminate prepared from the muscles of a calf.

Finally, BUCHNER tested the action of this proteid upon himself. One cubic centimetre of a very dilute solution, containing 3.5 milligrammes of the solid proteid, was injected under the skin of the forearm with antiseptic precautions. Two hours later there was marked pain along the lymphatics, especially localized in the elbow and axilla. The temperature showed no marked elevation (only 37.8°). On the following day there were marked erysipelatous redness and swelling extending for some inches about the place of injection, and accompanied by severe pain. The inflamed area felt hot, and projected distinctly above the surrounding surface. The lymphatics of the arm appeared like red cords. On the third day the swelling and redness were more marked, and extended from the wrist to the elbow. On the fourth day the symptoms began to recede. Here we have clinically a perfectly typical erysipelas with lymphangitis, and BUCHNER claims that all the cardinal symptoms of inflammation—rubor, calor, dolor—could not be produced without involvement of the solid tissues.

Similar, but less marked, symptoms were induced by the injection of a dilute solution of vegetable casein.

BUCHNER states that bacteria will not cause inflammation unless they be broken down. The pyogenetic substance contained within the bacterial cell can have no chemotactic action until the cell disintegrates. Thus, the anthrax bacillus contains a pyogenetic substance, but no pus is formed in mice with anthrax, because there is no destruction of the bacilli. This pyogenetic proteid of the anthrax bacillus, however, manifests its action in malignant pustule.

These experiments are of the greatest interest. We must say, however, that it is possible that the bacterial cellular proteid may be modified by the treatment to which it has been subjected in these experiments. We do not as yet know enough about the nature of this proteid to say that its nature and its action are not altered by being heated for hours with an alkali. However, accepting BUCHNER's work, it throws much light upon processes which have heretofore been but imperfectly understood.

THE SUMMER DIARRHŒAS OF INFANCY.—In a paper published in 1888, VAUGHAN stated that the microörganisms which produce the catarrhal or mucous diarrhœas of infancy are probably only putrefactive or saprophytic in character, and that they prove harmful by splitting up complex molecules and forming chemical poisons. At that time it was generally believed that a specific germ would be found, but the truth of the above statement is being made more manifest with every experimental study of the subject. Able and diligent bacteriologists, among whom BOOKER, in this country, and ESCHERICH, in Germany, deserve special mention, have made a careful study of the bacteria found in the intestines and stools in these diseases, and all agree that no specific organism has been found. BOOKER has reported the isolation of more than thirty kinds. In true cholera infantum the proteus group of bacteria was found in fifteen out of nineteen cases, but in the ordinary diarrhœas there is no constancy in the species present. Germs which are frequently found one year are rarely seen in the cases observed the next summer. This has been the experience of all who have studied the bacteria of the summer diarrhœas of infancy. VAUGHAN has studied the chemical products of the germs x, a, and A of Booker's list in the following manner and with the results as stated below.

Of these germs, **Booker** makes the following statements:

"x was found almost as a pure culture in the feces of a fatal case of diarrhœa. a was strongly pathogenic when tested last winter. A was isolated last summer; liquefies gelatin, and belongs to the proteus group."

Beef-tea cultures of each of these germs were made and kept in an incubator at 37° for forty-eight hours. At the expiration of this time these cultures were used for inoculating flasks of sterilized beef-broth. Eight flasks, each containing about ten ounces, were employed for each germ. These cultures were kept in the incubator at 37° for ten days. They were then twice filtered through heavy Swedish filter-paper. The second filtrate was allowed to fall into a large volume of absolute alcohol feebly acidified with acetic acid. A voluminous, flocculent precipitate resulted in each case. After the precipitates had subsided the supernatant fluid was decanted. The precipitates were then treated with distilled water, in which those from x and a were soluble, while that from A proved insoluble. A large volume of absolute alcohol was again added, and the mixture allowed to stand for four days. The precipitates from x and a completely subsided, leaving the supernatant fluids perfectly clear; but in the case of A the subsidence was not complete. The precipitates were collected, by decantation and filtration, on porous plates, and dried over sulphuric acid. These substances are proteid in composition, but differ from known proteids and from one another That from x is slightly yellow, as seen deposited in the alcohol, but becomes grayish on exposure to the air. It is readily soluble in water, from which it is not precipitated by heat or nitric acid, singly or combined.

It gives the biuret and xantho-proteid reactions. It is precipitated by saturating its aqueous solution with ammonium sulphate, and therefore cannot be classed with the peptones. Sodium sulphate and carbonic acid fail to throw it down from its aqueous solution, consequently we must say that it is not a globulin.

This leaves us with no other choice than to place it among the albumins, but we must admit that it possesses properties which do not belong to the known albumins.

The proteid prepared from cultures of the germ a is, as seen under the alcohol, very light, flocculent, and perfectly white, but so soon as it is brought in contact with the air it begins to blacken, and finally dries down on the porous plate in black scales.

It possesses the same general properties in regard to the action of solvents and other reagents which were found to be possessed by the proteid obtained from cultures of x.

The proteid of A is peculiar, inasmuch as it is practically insoluble in water.

These three proteids are highly poisonous. When injected under the skin of kittens or dogs they cause vomiting and purging, and, when employed in sufficient quantity, collapse and death. Post-mortem examination shows the small intestine pale throughout and constricted in places. The heart has been invariably, so far, found in diastole and filled with blood. The following brief notes from the record of experiments will illustrate the nature of the symptoms and the post-mortem appearances.

A small amount of proteid from bacillus x, dissolved in water, was injected under the skin on the back of a kitten about eight weeks old. Within one-half hour the animal began to vomit and purge, and death resulted within eighteen hours. The small intestines were pale, contracted in places, and contained a frothy mucus. The stomach was distended with gas and contained yellowish mucus. The liver was normal, the spleen and kidneys congested, and the heart distended.

Another kitten was treated with the proteid from bacillus a, dissolved in water. The vomited and fecal matters in this case were green. The animal died after fifteen hours, and presented appearances practically identical with those mentioned above.

A third kitten was treated with some of the proteid of bacillus A, suspended in water, and presented substantially the same symptoms and post-mortem appearances.

A fourth animal was treated in the same manner as the above with a proteid prepared from some canned meat. This was done as a control on the above experiments, and the kitten remained unaffected. This experiment demonstrates the fact that the poisonous properties are peculiar to the bacterial proteids.

Concerning the amount of one of these proteids necessary to produce a fatal result in the animals experimented upon a few experiments have been made.

Under the skin on the back of a guinea-pig, VAUGHAN injected ten milligrammes of the dry-scale proteid from bacillus a. This caused death within twelve hours. Of two kittens treated with fifteen milligrammes each of the a albumin, one died after forty-eight hours and the other recovered after two days of purging and vomiting. Two dogs, of about five pounds' weight, had each forty milligrammes, and, after serious illness of two days' duration, speedily recovered.

During these two days of vomiting and purging the dogs were constantly shivering, as with cold, but the rectal temperature stood at from 102.5° to 103.5° F.

There was in no case any sign of inflammation at the point of injection.

Plate cultures have been made from the proteids themselves and from the blood, liver, spleen, and kidneys of some of the animals killed with the proteid, and these plates have remained sterile, thus demonstrating that no germ has been introduced into the animal along with the chemical poison.

What conclusions may we draw from these facts when considered in connection with the results of the labors of BOOKER and ESCHERICH? We will formulate our ideas in the following propositions:

(1) There are many germs, any one of which, when introduced into the intestines of the infant, under certain favorable conditions, may produce diarrhœa.

As has been stated, many different germs have been found in the intestines of infants suffering from summer diarrhœa, and we now find that three species of these are capable of producing chemical poisons, which induce effects substantially identical with the symptoms observed in the infants, and it is not unreasonable to suppose that many other of these germs produce similar poisons.

(2) Many of these germs are probably truly saprophytic. A germ growing in the intestine does not necessarily feed upon living tissue. The food in the duodenum before absorption has no more vitality than the same material in the flask. Moreover, the excretions poured into the intestines from the body are not supposed to be possessed of vitality. A germ which will grow upon a certain medium in the flask and produce a poison will grow on the same medium in the intestine and produce the same poison, provided it is not destroyed by some secretion of the body.

(3) The only digestive secretion which is known to have any decided germicidal effect is the gastric juice; therefore, if the secretion be impaired there is at least the possibility that the living germ will pass on to the intestine, will there multiply, and will, if it be capable of so doing, elaborate a chemical poison which may be absorbed.

There is no longer any doubt that the acid of the gastric juice has a marked germicidal effect upon many of the microörganisms.

VAUGHAN has found that an exposure to a two-tenths per cent. solution of hydrochloric acid for half an hour will destroy Eberth's germ and two poison-producing bacilli which he has isolated from drinking-water which was believed to have caused typhoid fever. Although the germicidal effect of this acid has not been tried on the bacteria under consideration, doubtless it will be found to be considerable.

The chief reason why the breast-fed child has a better chance for life than the one fed upon cow's milk lies in the fact that the former gets its food germ-free; but a second reason is to be found in the larger amount of acid required to neutralize the cow's milk, as has been pointed out by

ESCHERICH. The gastric juice is the physiological guard against infection by way of the intestines.[1]

It is also possible that some of the secretions poured into the intestines have germicidal properties, or that the cells, in absorbing the poisonous proteids, may to a limited extent so alter them that they are no longer poisonous, or that in a perfectly normal condition the liver may be able to prevent these poisons from entering the general circulation without change. These are all possibilities, which science at some time in the future will investigate.

(4) Any germ which is capable of growing and producing an absorbable poison in the intestine is a pathogenic germ.

It is not necessary that a germ be capable of growing and causing disease and death when injected under the skin or into the blood in order to establish its right to rank with the pathogenic germs. In the blood the organism is acted upon by a wholly different fluid from that with which it is surrounded in the intestine, and the germicidal properties of the blood have been unquestionably demonstrated.

(5) The proper classification of germs in regard to their relation to disease cannot be made from their morphology alone, but must depend largely upon the products of their growth.

As has been stated, three microörganisms, differing sufficiently to be recognized as of different species, produce poisons, all of which induce vomiting and purging, and, when used in sufficient quantity, death. Morphologically these bacilli may not be closely related, but physiologically they are near akin.

If these deductions be true, we will try to avoid the introduction into the alimentary canal, not only of the so-called specific pathogenic germs, but of all toxicogenic microörganisms.

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[1] It has been said that this statement cannot be true, because there are other acids which are more powerful germicides than hydrochloric acid, but there is no force in this argument. The question is not whether the stomach is supplied with the very best germicide, but whether it is supplied with any at all. The human eye may not be a perfect mechanism, but it is man's only organ of vision.

Baginsky and Stadthagen have obtained from cultures of the "white liquefying bacterium" of the former a poisonous proteid which produces in mice, after about five hours, slight dyspnœa. The coat becomes rough, the animal sits with drooping head, and when forced to move does so sluggishly, but without any evidence of paralysis. The marked apathy increases, and death results after two or three days. Section shows an infiltration about the place of injection, congestion of the spleen, liver, and peritoneum. The intestine is hyperæmic throughout its entire length, and its upper portion contains a reddish-brown fluid.

From cultures of the same bacterium Baginsky and Stadthagen have also obtained a poisonous ptomaïne, which is probably identical with one found by Brieger in putrid horseflesh, and which has the formula $C_7H_{17}NO_2$.

That tyrotoxicon is one of the causes of the violent choleraic diarrhœa of children there can scarcely be a doubt. The symptoms induced by the poison cannot be distinguished from those of the disease. The post-mortem appearances are very much alike, if not identical, and the poison has been found in a sample of milk a part of which had been given to a child not more than two hours before the first symptoms of a violent attack of the disease made themselves manifest.

Typhoid Fever. — In 1880, Eberth discovered a bacillus which he believed to be the cause of typhoid fever, and this belief has been quite generally accepted. In the first edition of this work it was stated that the fever and the characteristic lesions of the disease had been produced in animals by inoculation with this germ. This is now known to be erroneous. As has been stated (page 93), the essential lesions of typhoid fever may be produced in animals with a number of microörganisms, among which, however, the Eberth bacillus is not included. The results obtained by Fränkel and Simmonds, and Seitz have been shown by Beumer and Peiper to be fallacious, and the germ with which the experiments were made by Vaughan and Novy, and mentioned in the first edition, is known not to be identical with that of EBERTH. It is true that this germ induced in dogs a continued fever of from twenty-eight to thirty-five days in duration, terminating in some instances fatally and revealing ulceration and perforation of the small intestines, but *for this reason* it is known to be different from EBERTH'S bacillus, because the latter never induces these effects. Notwithstanding this failure to affect the lower animals, the majority of bacteriologists believe, as has been stated, that the EBERTH bacillus is the sole and only cause of typhoid fever. In this believe VAUGHAN refuses to concur, and claims that the EBERTH bacillus as found in the spleen after death is an involution form of any one of a number of germs which are found in certain waters. As this is not the place for an extended discussion of purely morphological questions, the reader is referred to the literature of the subject, and we will content ourselves with giving the following summary of what is known concerning the chemical products of the EBERTH bacillus and of the germs studied by VAUGHAN.

In 1885, BRIEGER obtained from pure cultures of the EBERTH bacillus a poisonous ptomaïne, which produced in guinea-pigs a slight flow of saliva, frequency of respiration, dilatation of the pupils, profuse diarrhœa, paralysis, and death within from twenty-four to forty-eight hours. Post-mortem examination showed the heart in systole, the lungs hyperæmic, and the intestines contracted and pale. At first BRIEGER was inclined to regard this as the specific poison of typhoid fever and named it typhotoxine. However, he has more recently modified his opinion and is inclined to regard typhoid fever as due to a mixed infection.

BRIEGER and FRÄNKEL have found in cultures of the EBERTH bacillus a proteid which causes death in rabbits after from eight to ten days. They say nothing about the symptoms.

In 1889, VAUGHAN isolated from mixed cultures from typhoid stools a base, forming crystalline salts and capable of inducing in cats and dogs a marked elevation of temperature accompanied by severe purging. The following is the record of one experiment with this substance: "An aqueous solution of the crystals was given to a dog by the mouth at 3 P.M. The rectal temperature before the administration was 101° F. At 3.15, retching and vomiting set in and continued at intervals for more than two hours. At 3.30, the temperature was 103° F. At 3.55, the animal began to purge. The first discharges contained much fecal matter, but subsequently they were watery and contained mucus plainly stained with blood. At 4, the temperature was 103.5° F. and remained the same at 4.30. The animal was not seen again until 10 A.M. the next day, when its temperature was 100.5°, and recovery seemed complete."

This base was not obtained in quantity sufficient for an ultimate analysis. The platino-chloride crystallizes in fine rhombic prisms and the hydrochloride in long, delicate, red needles. The red color seems to be inherent to the substance and not due to impurities. The mercury and platinum compounds are insoluble in alcohol, soluble in water. The hydrochloride is soluble in both water and alcohol.

In 1890, VAUGHAN reported the isolation, from water supposed to cause typhoid fever, of a number of toxicogenic germs. The chemical products of two of these have been studied. They belong to the proteids, and an analysis of one of them by FREER shows it to belong to the nucleins. These poisons are soluble in water, the opalescent solution showing a distinctly acid reaction. They are not precipitated by heat or nitric acid singly or combined. They dissolve in nitric acid, forming a colorless solution, which becomes yellow on the addition of ammonia. They dissolve in caustic alkalies and the solution becomes purple on the addition of a dilute solution of copper sulphate.

On white rats these poisons produce symptoms which are identical with those which follow inoculations with the living germs. The rat seems to shiver with cold and gives evidence of abdominal pain. It lies with its limbs flexed and head drawn down for a few seconds, then stretches out the limbs. It lies on the side for a short time, then sits with the head drawn under the body.

Dogs shiver as with cold, but at the same time the rectal temperature is from one to four degrees above the normal. In some instances vomiting and purging have been induced.

The following experiments seem to show that the poison accumulates in the nerve-centres :

Two guinea-pigs were treated with hypodermic injections of one of these poisons, the amount used being about ten times the dose which ordinarily proves fatal to these animals. Within twelve hours both were dead. Plate cultures made from the liver, spleen, blood, brain, and spinal cord remained sterile. Small quantities of the brain and cord were rubbed up in a sterilized dish with sterilized water, and two c. c. of the emulsion were injected under the skin of each of four guinea-pigs. These animals seemed to be very excitable the next day, throwing themselves about violently in the cages when slight noises were made near them. Within a period of from sixteen to twenty-four days all died. This experiment needs repetition, and it will be necessary to prepare and inject similar emulsions made from other organs before any positive conclusions can be drawn.

In a study of fatal cases of typhoid fever at Bucharest BABES finds that the typical germ differs markedly from that of EBERTH.

SWINE-PLAGUE, OR HOG-CHOLERA.—The researches of LÖFFLER, SCHÜTZ, LYDTIN, and SCHOTTELIUS in Europe, and of BILLINGS and SALMON in this country, have demonstrated the existence among swine of at least three infectious diseases. These are—

(1) Hog-erysipelas, or rouget of France, or Schweinerothlauf of Germany.

(2) German swine-plague, or Schweineseuche.

(3) American swine-plague (BILLINGS), or hog-cholera (SALMON).

The first two of these are exclusively European diseases, and their chemical poisons have not been studied.

The American swine-plague is preëminently a disease of the digestive tract involving most markedly the large intestine. It is the great swine disease of this country, and is probably present in England, where it is associated with other diseases under the name of swine-fever. A disease which was observed in Denmark and Sweden for the first time in 1888–89 and known as swine-pest or swine-diphtheria, has been shown by SELANDER, FROSCH, and others to be identical with our swine-plague. In the summer of 1889 France was visited by a swine disease, which is considered by CORNIL and CHANTEMESSE to be identical with the German swine-plague, but which RIETSCH and JOBERT, after a comparative study of the microörganisms, pronounce as the American disease. In this country we have at present no positive demonstration of the existence of any other infectious swine disease. The swine-plague of SALMON has been the subject of considerable discussion, but its existence can hardly be said to be established.

The following statements concerning the chemical poisons refer to the swine-plague of BILLINGS or the hog-cholera of SALMON, which are only two names for one disease.

In pure cultures of this bacillus NOVY has found a poisonous base, which probably has the composition $C_{10}H_{26}N_2$, and to which he has provisionally given the name, susotoxine. One hundred milligrammes of the hydrochloride of this base causes in white rats convulsive tremors and death within one and one-half hours. Post-mortem examination shows the heart in diastole, lungs pale, stomach contracted, a serous effusion in the thoracic cavity, and the subcutaneous tissue pale and œdematous.

NOVY has also obtained a poisonous proteid from cultures of this germ. The following experiments illustrate the effects obtained with this body: 100, 50, and 25 milligrammes, respectively, were injected into three young rats from the same litter. The animal which received 100 mg. soon began to crawl about on its belly, being unable to rise. The eyes were soon filled with a thick secretion and the toes became red. Finally it became quiet, lying on its belly, with feet extended. The respirations became deeper, and a coma-like condition set in. The animal died, without convulsions, within about three hours. The rat which received 50 mg. went through the same course of symptoms, but these were less intense. Death resulted four hours after the injection. The one which received 25 mg. became very sick, but finally recovered, and one week later it was given another injection of 30 mg., which produced scarcely any effect. Then it was treated at intervals of five, three, five, two, and four days, respectively, to 40, 50, 75, 100, and 125 mg. without effect. Three days after the last injection the animal was inoculated with one c. c. of a bouillon culture of the highly virulent germ. Only a slight temporary effect was observed during the first day, after which recovery was complete and permanent. A control rat which was given the same quantity of the culture sickened the next day and died one week later. From this it will be seen that the animal was rendered immune against the disease.

SCHWEINITZ also reports the detection of a slightly poisonous base, which he designates as sucholotoxin, and a poisonous proteid, and with these he has been able to secure immunity in guinea-pigs against the virulent germ. The proteid body is classed among the albumoses, and is said to crystallize in white, translucent plates when dried in vacuo over sulphuric acid and to form needle-like crystals with platinum chloride. No one else has reported a crystalline bacterial proteid, and this body is deserving of a more extended study.

RABBIT SEPTICÆMIA.—HOFFA has killed rabbits by inoculation with pure cultures of the bacillus of this disease, and has isolated from the bodies of these animals methylguanidin, while in the bodies of healthy rabbits this poison could not be found. The fatal dose of methylguanidin for rabbits was found to be 0.2 gramme when given subcutaneously. Since HUEPPE has suggested that the bacterium of chicken-cholera is identical with that of rabbit septicæmia, chickens were poisoned with methylguanidin, and the symptoms were observed to be analogous to those of the disease.

PNEUMONIA.—BONARDI has made a chemical study of the diplococcus of FRÄNKEL. He finds certain poisons—ptomaïnes—which he has been unable as yet to obtain in quantity sufficient for ultimate analysis. He also claims to have secured immunity against the germ by treating rabbits with small quantities of the chemical poisons.

MALIGNANT ŒDEMA.—KERRY finds that the bacillus of this disease decomposes albumin with the formation of fatty acids, leucin, hydro-paracumaric acid, and a foul-smelling oil of the composition $C_8H_{16}O_4$. This oil is insoluble in water, alkalies, and acids, easily soluble in ether, benzol, bisulphide of carbon, and alcohol. It is optically inactive, and on being oxidized furnishes valerianic acid. Nothing is said concerning its action upon animals. Among the gaseous products are carbonic acid, hydrogen, and marsh gas. The author was unable to determine whether or not free nitrogen is formed.

PUERPERAL FEVER.—BOURGET claims to have isolated several ptomaïnes from the urine of women with puerperal fever. His conclusions are as follows: (1) In puerperal fever the urine contains highly poisonous bases. (2) The toxicity of the urine is most marked when the symptoms of the disease are most grave, and diminishes as the symptoms abate. (3) The ptomaïnes obtained from the urine prove fatal when injected into frogs and guinea-pigs. (4) Toxic bases, resembling those obtained from the urine, were extracted from the viscera of a woman who had died of puerperal fever.

CHAPTER VI.

THE NATURE OF IMMUNITY-GIVING SUBSTANCES.

OGATA and JASUHARA find that anthrax bacilli grown in the blood-serum of animals naturally immune to the disease will not on subsequent inoculation induce the disease in animals naturally susceptible. Thus, anthrax germs grown in frog-blood make mice sick, but do not prove fatal to them, and those grown on the blood-serum of white rats or dogs have a similar effect upon rabbits; but germs grown in the blood of animals not immune kill both mice and rabbits. They also find that the injection of one drop of frog blood-serum or one-half drop of serum from a dog into a mouse, any time within seventy-two hours before to five hours after inoculation with anthrax, protects this animal from the disease. A guinea-pig weighing 400 grammes was given twenty drops of frog's blood diluted with the 0.6 per cent. salt solution and immediately thereafter inoculated with virulent anthrax; the animal became slightly sick, but soon recovered. The same was true of a rabbit weighing 1500 grammes which was treated with 8 c.c. of defibrinated dog's blood. The experimenters conclude that one-fourth of a drop of the serum of the dog diluted to three times its volume with the salt solution is the smallest amount which will give immunity against anthrax to a mouse of 10 grammes.

KITASATO and BEHRING have secured immunity in some animals against tetanus and diphtheria by the following methods:

(1) By the method of FRÄNKEL (for diphtheria), which has been given. (See page 128.)

(2) By the addition of iodine trichloride to cultures four weeks old, in the proportion of 1 : 500; allow to stand for sixteen hours; inject 2 c.c. into the abdominal cavity of a

guinea-pig; three weeks later inject 0.2 c.c. of a culture in bouillon containing iodine trichloride in the proportion of 1 : 5500.

(3) By the metabolic products of the diphtheria bacillus in the living body. In the pleural cavities of guinea-pigs killed by inoculation with the germ there is often a reddish, germ-free transudate; 10 c.c. to 15 c.c. of this kills guinea-pigs; small amounts give immunity.

(4) By inoculating with the virulent germ and arresting the growth of the same with iodine trichloride, gold-sodium chloride, naphthylamine, or carbolic acid. Of eight guinea-pigs, each of which was inoculated with 0.3 c.c. of a virulent culture, two, which were not treated, died within twenty-four hours; four, which had—two each—a 1 per cent. and a 2 per cent. solution of iodine trichloride injected immediately and at the place of inoculation, recovered; of two which had the same treatment six hours after the inoculation, one died after four days.

(5) By peroxide of hydrogen in diluted sulphuric acid. Guinea-pigs bear from 1 : 4000 to 1 : 2500; mice, 1 : 2000 to 1 : 800; rabbits, less than 1 : 1500 of this substance per body-weight. Injections of this solution before inoculation give more or less immunity, or, rather, increase the resistance to the disease; given after inoculation it hastens death.

None of these methods are applicable to the prevention or treatment of the disease in man.

Tizzoni and Cattani have reviewed the above statements in so far as they refer to tetanus. These experimenters find that the addition of an equal volume of either a 2 per cent. solution of fresh chlorine water or iodine trichloride, or a 5 per cent. solution of phenylic acid to the poisonous, filtered tetanus culture destroys the toxicity of the same; but they state that the injection of these substances into animals either before or after inoculation with the germ has no effect upon the development or course of the disease.

However, they do find that the blood-serum of an animal which is immune will protect against either the living germ or the germ-free culture. Pigeons and dogs are but slightly susceptible to tetanus and they are made still less so by being treated for a number of times with small quantities of the virulent culture. After each recovery these animals are found to be less susceptible, and finally they acquire a high degree of immunity, and then their blood is employed in securing immunity in other animals much after the manner already detailed for anthrax.

TIZZONI and CATTANI have attempted to ascertain the nature of that constituent of the blood-serum which gives immunity. In these experiments serum from a dog which had been rendered immune against tetanus was employed. In the first place, a filtered culture of the tetanus germ was concentrated in vacuo at 40° until one-half c.c. of it would kill a rabbit within thirty-six hours. To this amount of the culture, the blood-serum was added after having been subjected to varied treatment, and the whole was subsequently injected into a rabbit. The blood-serum retains its antitoxic properties when kept in the dark at 15° for some days, and it may be heated to 60° without injury. A temperature of 65° weakens, and one of 68° (the temperature at which the serum coagulates) completely destroys the antitoxic properties of the serum. The "tetanus-antitoxine" is non-diffusible. It is precipitated from the blood-serum on the addition of absolute alcohol and from the dried percipitate it may be extracted either with water or glycerin, though very slowly with the latter. From these facts it is concluded that the antitoxin is a proteid with the characteristics of an enzyme.

HANKIN gives the following argument in favor of the theory that immunity is not due to ptomaïnes, but to proteids: "It is generally admitted that in acquired immunity against a disease we are dealing (for the most part, at least) with a phenomenon of the nature of acquired tolerance of a poison. If we consider what this theory really implies, and, further, suppose that the poison involved is a ptomaïne or other body of an alkaloidal nature, numerous difficulties immediately present themselves. For, in the first place, if acquired immunity is of this nature, we are dealing with an acquired tolerance of a poison, which tolerance is conferred by administering a single dose, or at most a very limited number of doses. Further, this acquired tolerance, thus easily obtained, is very permanent, lasting for months, or even years. Now, though acquired tolerance of alkaloids is constantly observed, it is but limited in degree, and only obtained as the result of a long-continued succession of doses.[1] Secondly, since acquired tolerance of this hypothetical poison results in the microbe being no longer capable of living in the body, this theory implies that the poison in question is one that is produced by the microbe in order to live there. In other words, that it is a poison capable of lowering the bacteria-killing power possessed by every living animal body.[2]

"Of course, it is conceivable that a ptomaïne might be concerned in doing this, but, so far as I know, no parallel to such action can be found among bodies of an alkaloidal nature.

"When, however, we turn to what is known of poisonous proteids, we at once find that they have properties analogous to those of the hypothetical immunity-giving poison.

"First, as regards the question of tolerance: Two poisons are known, which, in the nature of the tolerance they produce, resemble the hypothetical poison in question. Both of them are albumoses. The first is the ordinary hemialbumose of proteid digestion. It is known that the injection of a single minute dose confers immunity against a further dose for a period of twelve hours. The second albumose is the poisonous principle of snake-poison. Sewall, in 1887, published a very interesting research on acquired immunity against snake-poison. He showed that it was possible, by the injection of a few minute doses, to give pigeons such a tolerance of this substance that, three months after the treatment, they were able to stand what would otherwise be seven times the lethal dose. He suggests in his paper that, by inoculation with the ptomaïnes produced by bacteria, it may be possible to protect animals against their disease-producing powers, although the remarkable case of tolerance he had discovered suggested that not ptomaïnes, but albumoses, were the substances concerned in giving immunity against a disease;[1] for I suggest that this fact—that the only cases of tolerance known which resemble the tolerance implied in disease-immunity are cases of tolerance against albumoses—strongly suggests that immunity against a disease is immunity against an albumose produced by the microbe."

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[1] CARBONE claims to have obtained immunity in rabbits against the action of the proteus vulgaris by means of not more than two previous injections of small quantities of neurin obtained from cultures of the proteus. He still further states that immunity against the same germ is obtained by muscarin, which produces physiological effects practically identical with those of this neurin.

[2] With this statement we must take issue. The experiments already given in which immunity is induced in a susceptible animal by the injection of the serum of the blood of an animal naturally immune show that the immunity-giving substance is not necessarily of bacterial origin, and certainly that it is not necessarily a product of the germ against which the immunity is secured.

In conformity with the above-stated theory, Hankin prepared, as we have already stated, from cultures of the anthrax bacillus a poisonous albumose, which, when employed in small doses, gives immunity; in large doses, proves fatal. Hankin endeavored to separate any ferment that might be present and to which the immunity might possibly be due. A quantity of lime-water was added to a solution of the albumose and the lime precipitated by the addition of phosphoric acid. Theoretically, the precipitate should contain any ferment present, and the immunity-giving property of the albumose would be diminished by the amount of the ferment thus removed, in case the immunity be due to the ferment. However, the albumose was found to have lost none of its immunity-producing power. From this Hankin concludes that the albumose is the real immunity-producing agent. He does not in-form us whether or not any test of the phosphate precipitate was made.

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[1] We would suggest the fact that in 1887 it was not known that bacteria produce albumoses, and at that time the term "ptomaïne" was employed to indicate all the bacterial poisons.

BACTERIAL PRODUCTS WHICH FAVOR THE DEVELOPMENT OF INFECTIOUS DISEASES.—ROGER has made a very interesting contribution on this subject, and if his work be confirmed the question of mixed infection will become more important than it has been supposed to be. Rabbits are not naturally susceptible to the germ of charbon symptomatique; indeed, inoculation with pure cultures of the bacillus has no visible effect. But ROGER finds that if the staphylococcus pyogenes aureus, proteus vulgaris, or bacillus prodigiosus be injected into the animal at the same time with the germ of charbon symptomatique the latter develops and produces the disease. The same result is obtained when a sterilized culture of the bacillus prodigiosus is employed. He at first supposed that the chemical products of the bacillus prodigiosus so lowered the vitality of the tissues that the pathogenic germ was enabled to establish itself; but he found that the same results were obtained when the two inoculations were made in distant parts of the body. The most marked effects were seen when the sterilized culture was injected into a vein and the charbon bacillus subcutaneously. In these instances the rabbits rapidly developed enormous tumors, and died within twenty-four hours. One drop of the sterilized culture was found to be sufficient, when injected intravenously, to render rabbits susceptible to the pathogenic germ.

In this connection it may be remarked that from time to time statements have been made which would lead us to infer that there are certain poisonous proteids which in some way yet unknown render the body especially susceptible to the invasion of bacteria. ROSSBACH injected a poisonous albumose from the juice of the papain tree into the bloodvessels of animals and obtained a septicæmia. The blood was found to be filled with non-pathogenic germs which came from the intestines. The results of ROSSBACH have, however, been questioned by others. HANKIN makes the statement that a small dose of snake-poison, which is too small to kill the animal outright, after a certain time may cause death from septicæmia. He says: "The albumose of the snake-poison has apparently so far suppressed the germicidal power of the animal that ordinary decay-producing bacteria can increase and multiply in the blood. Further, it is often remarked that animals killed by a snake-bite putrefy rapidly, as if the bacteria-killing power of the blood-serum had been diminished." A similar statement has been made concerning the action of the poisonous albumose of jequirity seeds. Further investigation must discover how much of truth and how little of error lie in these claims.

CHAPTER VII.

THE GERMICIDAL PROTEIDS OF THE BLOOD.

As early as 1872 Lewis and Cunningham showed that bacteria injected into the circulation rapidly disappear. In the blood of twelve animals, which had been treated with such injections, bacteria could be found in only seven after six hours. In thirty animals, bacteria were found in the blood of only fourteen after twenty-four hours, and in seventeen animals, bacteria were found in only two when the examination was made from two to seven days after the injection.

In 1874, Traube and Gscheidlen found that the blood taken from a rabbit into the jugular vein of which forty-eight hours before 1½ c.c. of a fluid rich in putrefactive germs was injected, remained without undergoing decomposition for months. These investigators attributed the germicidal properties of the blood to its ozonized oxygen. Similar results were obtained by Fodor and Wysokowicz. The latter accounted for the disappearance of the bacteria not by supposing that they were destroyed by the blood, but that they found lodgement in the capillaries.

The first experiments made with extra-vascular blood were conducted by Grohmann under the direction of A. Schmidt. It was found that anthrax bacilli, after being kept in coagulating plasma, were less virulent, as shown by their effects upon rabbits. Grohmann supposed that in some way the bacteria were influenced by the process of coagulation.

In 1887, Fodor made a second series of experiments in which he used blood taken from the heart, and showed the marked germicidal properties of this on anthrax bacilli.

In 1888, Nuttall used defibrinated blood taken from various species of animals (rabbits, mice, pigeons, and sheep) and found that this blood destroyed the bacillus anthracis, bacillus subtilis, bacillus megaterium, and staphylococcus pyogenes aureus when brought in contact with them. NISSEN continued this work and employed blood-serum as well as defibrinated blood. The conclusions reached were as follows:

(1) The addition of small quantities of sterilized salt-solution or bouillon to the blood does not destroy its germicidal properties.

(2) Cholera germs and EBERTH's bacilli are easily destroyed by fresh blood.

(3) For a given volume of blood there is a maximum amount of bacilli which can be added. If too many germs are used the destruction is incomplete.

(4) Blood whose coagulability has been destroyed by the injection of peptone is still germicidal.

(5) Filtered blood-plasma from the horse is germicidal.

BEHRING has attributed the action of the blood of white rats on anthrax bacilli to the presence of a hypothetical basic body to which the decidedly alkaline reaction of the blood is supposed by him to be due. Later, he lays special stress upon the amount of carbonic acid gas in the blood-serum.

BUCHNER has made a most exhaustive study of this subject, in which he has been aided by VOIT, SITTMANN, and ORTHENBERGER. The results of this work are stated as follows:

(1) The germicidal action of the blood is not due to phagocytes, because it is not influenced by freezing and thawing the blood, by which the leucocytes of the blood of the rabbit are destroyed.

(2) The germicidal properties of the cell-free serum must be due to soluble constituents.

(3) Neither neutralization of the serum, nor the addition of pepsin, nor the removal of carbonic acid, nor treatment with oxygen have any effect upon the germicidal properties of the blood.

(4) Dialysis of the serum against water destroys its

activity, while dialysis against 0.75 per cent. salt solution does not. In the diffusate there is no germicidal substance. The loss by dialysis with water must be due to the withdrawal of the inorganic salts of the serum.

(5) The same is shown to be the case when the serum is diluted with water and when it is diluted with the salt solution. In the former the germicidal action is destroyed, while in the latter it is not.

(6) The inorganic salts have in and of themselves no germicidal action. They are active only in so far as they affect the normal properties of the albuminates of the serum. The germicidal properties of the serum reside in its proteid constituents.

(7) The difference in the effects of the active serum and that which has been heated to 55° is due to the altered condition of the albuminates. This difference may possibly be a chemical one (due to changes within the molecule), or it may be due to alterations in mycelial construction. The albuminates act on the bacteria only when the former are in an "active state."

HALLIBURTON has prepared from the lymphatic glands a globulin which he designates as cell-globulin β, and which agrees with fibrin ferment in inducing coagulation in plasma. HANKIN has tested the germicidal properties of this cell-globulin. His experiments have been conducted in the following manner: The lymphatic glands (in later experiments the spleen, also) of a dog or cat are freed as much as possible from fat and connective tissue, then finely divided, and extracted with a dilute solution of sodium sulphate (one part of a saturated sodium sulphate solution + nine parts of water). The cell-globulin passes into solution, while the other proteids are but sparingly soluble. After twenty-four hours the fluid is filtered and mixed with an excess of alcohol. The voluminous precipitate containing the cell-globulin is collected on a filter and washed with absolute alcohol. For use, a part is dissolved in water and a small quantity of a bouillon culture of the anthrax bacillus added. Plate cultures are made along with control plates from time to time, and in this way the germicidal property of the substance is demonstrated. HANKIN closes this contribution with the following conclusions:

(1) HALLIBURTON's cell-globulin β has marked germicidal properties.

(2) In this respect it differs from fibrin ferment.

(3) The germicidal property of this substance seems to be identical with that of serum as described by BUCHNER, NISSEN, and NUTTALL.

(4) The active properties of the serum are probably due to this or to an allied body.

In a more recent contribution HANKIN designates the germicidal agents of the body as "defensive proteids." He thinks it probable that blood-serum owes its activity to these bodies and that the assumption of an "active condition of the serum albuminate" made by BUCHNER is unnecessary. He also thinks that BEHRING's supposed alkaline base exists in the form of an albumose. We know of three albumoses which are alkaline in reaction. These are the protomyosinose and deuteromyosinose of KÜHNE and CHITTENDEN, prepared by the digestion of myosin, and the anthrax albumose of MARTIN.

By a method similar to that which he had employed in the preceding experiments, HANKIN has isolated a "defensive proteid" from the blood-serum and the spleen of the rat. This substance belongs to the globulins, and the natural immunity of the rat against anthrax is probably due to its existence in the blood.

STERN finds that the blood taken from different men, or from the same man at different times, varies markedly in its germicidal properties; also, that the germicidal properties of the blood when kept at 42° are at least as great as at the normal temperature of the body. These statements are substantially confirmed by ROVIGHI.

CHAPTER VIII.

METHODS OF EXTRACTING PTOMAÏNES.

FROM what has been given in the preceding pages, one may gather some idea of the peculiar difficulties with which the chemist has to contend in his endeavors to isolate the basic products of putrefaction. He has to deal with very complex substances, of the nature and reactions of many of which he must be ignorant. Besides, the substances which he seeks are often most prone to undergo decomposition, and in this way escape detection. Many ptomaïnes are volatile or decomposable at any temperature near that of boiling water. In these cases, solutions cannot be evaporated in the ordinary way and the poison separated from the residue. Indeed, the investigator has frequently been disappointed when on the evaporation of a solution, which he has demonstrated to be poisonous, he finds that the residue is wholly inert. Again, he may destroy the ptomaïne by the action of reagents which he uses. So simple a procedure as the removal of a metallic base from a solution containing a ptomaïne, by precipitation with hydrogen sulphide gas, has been known to destroy wholly the ptomaïne. Probably the most perplexing difficulty in the isolation of these putrefactive alkaloids lies in the great number, complexity, and diversity of the other substances present in the decomposing mass. The same ptomaïne may be present in equal quantities in two samples of milk, and yet it may be easily obtained from the one, while from the other only minute traces can be secured. The difference is due to the fact that the other constituents of the milk in the two samples are at different stages of the putrefactive process, and, consequently, differ greatly in their reactions and in their effects upon the agents employed to isolate the poison. All chemists will appreciate these difficulties.

One of the first things for the chemist who undertakes to do this work is to ascertain whether or not his reagents are pure. We have found a number of samples of German ether, which was imported on account of its supposed purity, to yield on spontaneous evaporation a residue which gave several of the alkaloidal reactions, and a few drops of which, injected under the skin of a frog, caused paralysis and death within a few minutes. We would advise that 500 c.c. of the ether to be used should be allowed to evaporate spontaneously, and its residue, if there be one, be examined both chemically and physiologically. The basic substance which exists in some samples of sulphuric ether is pyridine.

GUARESCHI and MOSSO found commercial alcohol almost invariably to contain small quantities of an alkaloidal substance, the odor of which is similar to that of nicotine and pyridine. Its solutions are precipitated by gold chloride, phosphowolframic acid, phosphomolybdic acid, potassium iodide, and MAYER's reagent, but not by platinum chloride or tannic acid. It does not reduce, or reduces feebly, ferric salts. From one sample of alcohol they obtained a base which, in addition to the above reactions, did give a precipitate with platinum chloride. Alcohol may be freed from these substances by distillation over tartaric acid.

In amylic alcohol, HAITINGER has found as much as 0.5 per cent. of pyridine. It may be purified in the same manner as recommended for ethylic alcohol.

Chloroform, when found to leave any residue on evaporation, should be washed first with distilled water, then with distilled water rendered alkaline with potassium carbonate, then dried over calcium chloride and distilled.

Petroleum ether sometimes contains a base which has an odor similar to trimethylamine or pyridine, and which gives a precipitate with platinum chloride, forming in octahedra.

Benzole may contain a similar substance.

The following methods have been used for the purpose of extracting the putrefactive alkaloids:

THE STAS-OTTO METHOD.—This method depends upon the following facts: (1) The salts of the alkaloids are soluble in water and alcohol, and generally insoluble in ether, and (2) the free alkaloids are soluble in ether, and are removed from alkaline fluids by agitation with ether. These principles are capable of great variety in their application. The usual directions are as follows: Treat the mass under examination with about twice its weight of pure 90 per cent. alcohol, and from ten to thirty grains of tartaric or oxalic acid; digest the whole for some time at about 70°, and filter. Evaporate the filtrate at a temperature not exceeding 35° either in a strong current of air or in vacuo over sulphuric acid. Take up the residue with absolute alcohol, filter, and again evaporate at a low temperature. Dissolve this residue in water, render alkaline with sodium bicarbonate, and agitate with ether. After separation remove the ether with a pipette, or by means of a separator, and allow it to evaporate spontaneously. The residue may be further purified by redissolving in water and again extracting with ether.

The following modifications of this method are employed: Instead of tartaric or oxalic acid, acetic acid is frequently used.

When the fluid suspected of containing a ptomaïne is already acid from the development of lactic or other organic acid, the addition of an acid is often dispensed with.

Ether extracts are made from both acid and alkaline solutions.

Chloroform, amylic alcohol, and benzine are used as solvents after extraction with ether.

The modification of this method, as carried out by SELMI and MARINO-ZUCO is given in detail as follows:

The material is divided as minutely as possible, placed in a large flask, and treated with twice its volume of 90 per cent. alcohol, and acidulated with tartaric acid in the proportion of 0.5 gramme to 100 c.c. of the mixture, taking care from time to time that the reaction is permanently acid. The flask, which is connected with a reflux condenser, is now placed on the water-bath and kept at the constant temperature of 70° for twenty-four hours. While yet warm the liquid is transferred to a special apparatus for filtration by the aid of atmospheric pressure. The liquid is poured upon a wet cloth supported upon a perforated porcelain funnel, which is connected below with a receiver exhausted by a water-pump or aspirator. In this way rapid filtration is secured, and by repeated washing the extraction is made thorough. The acid alcoholic liquid is now transferred to a special distillation apparatus.

A large tubulated retort of ten litres capacity is connected by means of a cork to a large tubulated receiver. The tubulure of the retort is provided with a small perforated cork, which carries a glass tube finely drawn out and extending to the bottom of the retort. The tubulure of the receiver is connected with LIEBIG's bulbs containing dilute sulphuric acid (1 to 10), and the bulbs in turn are connected with a water-pump or aspirator.

In order to prevent the passage of air through the corks, they are covered with animal membrane which has been freed from fat. By means of the aspirator a fine current of air is drawn through the liquid and suffices to keep it constantly agitated. The retort is kept on the water-bath at a temperature of from 28° to 30°. The receiver is kept cold by a current of water In this manner the distillation of the alcohol goes on rapidly and conveniently. Moreover, decomposition is so far prevented that volatile bases are never found in the bulbs.

The aqueous residue, after the removal of the alcohol by distillation, is filtered and extracted with ether as long as anything is dissolved. It is then mixed with powdered glass and evaporated to dryness in vacuo. This residue is repeatedly extracted with absolute alcohol. The alcohol is distilled again in the apparatus already described. The residue is taken up with distilled water and filtered. It is then made alkaline with sodium bicarbonate and repeatedly extracted with ether, benzine, and chloroform.

In order to obtain the base from the solvent, the greater part may be evaporated on the water-bath and the remainder allowed to evaporate spontaneously, or the remainder may be treated with dilute hydrochloric acid and the evaporation continued on the water-bath or in vacuo.

DRAGENDORFF'S METHOD.—The finely divided substance is digested for some hours with water acidulated with sulphuric acid at from 40° to 50°. This is repeated two or three times, and the united filtered extracts are evaporated to a syrup. This is treated with four volumes of alcohol and digested for twenty-four hours at 30.° After cooling, the alcoholic extract is filtered, the residue washed with 70 per cent. alcohol, and the united filtrates freed from alcohol by distillation. The aqueous residue, diluted if desirable, is filtered and submitted to the following extractions:

(1) The acid liquid is shaken with freshly rectified petroleum ether as long as this reagent leaves any residue on evaporation.

(2) The acid fluid is now extracted with benzine.

(3) The next solvent used is chloroform.

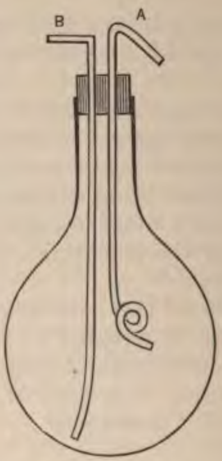
(4) The liquid is now again extracted with petroleum ether in order to remove traces of benzine and chloroform.

(5) The liquid is now made alkaline with ammonia and successively extracted with petroleum ether, benzine, chloroform, and amylic alcohol.

(6) The remainder of the ammoniacal liquid is mixed with powdered glass, evaporated to dryness, the residue pulverized, and extracted with chloroform.

The residue obtained with each of the above solvents should be examined for ptomaïnes.

BRIEGER'S METHOD.—The substance under examination is divided as finely as possible, and then heated with water slightly acidified with hydrochloric acid. During the heating care must be taken that the feebly acid reaction is maintained. The heating should continue for only a few minutes. The liquid is then filtered and concentrated, at first on a plate and then on the water-bath, to a syrup. If one has material which is highly odorous, as is the case frequently both with aqueous and alcoholic extracts of putrid material, BRIEGER recommends that a piece of apparatus devised by BOCKLISCH be used. The fluid to be evaporated is placed in a globular flask, the rubber stopper of which carries two small glass tubes. One of these, B, extends to the bottom of the flask, while A terminates just above the surface of the liquid. The tube, A, is connected with a water-pump or aspirator, which draws the vapor through the tube. In order to prevent the return of condensed fluids, the end of A in the flask is curved upon itself. The tube, B, is finely drawn out and through it a current of air is constantly moving. This prevents the formation of a deposit or a pellicle in the fluid. By regulating the amount of air coming through this tube, more or less of a vacuum will be formed in the flask. After evaporation to a syrup, an extraction is made with 96 per cent. alcohol, and the filtered extract is treated with a warm alcoholic solution of lead acetate. The lead precipitate is removed by filtration, the filtrate evaporated to a syrup and again extracted with 96 per cent. alcohol. The alcohol is driven off; the residue taken up with water; traces of lead removed with hydrogen sulphide; and the filtrate, acidified with hydrochloric acid, evaporated to a syrup. This syrup is extracted with alcohol, and the filtrate pre-



cipitated with an alcoholic solution of mercuric chloride. The mercury precipitate is boiled with water, and on account of differences in solubility of the double compounds with mercury, one ptomaïne may be separated from others at this stage of the process. (If thought best, the lead precipitate may be freed from lead and carried through the following steps of the process. BRIEGER has found small amounts of ptomaïnes in the lead precipitate only in his work with poisonous mussels.)

The mercury filtrate is freed from mercury, evaporated, and the excess of hydrochloric acid carefully neutralized with soda (the reaction is kept feebly acid), then it is again taken up with alcohol in order to free it from inorganic salts. The alcohol is evaporated, the residue taken up with water, the remaining traces of hydrochloric acid neutralized with soda; the whole acidified with nitric acid, and treated with phosphomolybdic acid. The phosphomolybdate double compound is separated by filtration, and decomposed by neutral acetate of lead. This is hastened by heating on the water-bath. The lead is removed by hydrogen sulphide, the filtrate is evaporated to a syrup and taken up with alcohol, from which many ptomaïnes are deposited as chlorides, or double salts may be formed in the alcoholic solution. BRIEGER states that the chlorides as deposited from the alcoholic solution are seldom pure, and he advises for their purification, precipitation with gold chloride, platinum chloride, or picric acid, and, on account of differences in solubility of these double salts, the process of purification is rendered more easy. The chloride of the base is obtained by removing the metallic base with hydrogen sulphide; while the picrate is taken up with water, acidified with hydrochloric acid, and repeatedly extracted with ether, in order to remove the picric acid.

THE METHODS OF GAUTIER AND ETARD.—The putrid matters, liquid and solid, are distilled at a low temperature in vacuo. The distillate (A) contains a considerable quantity of ammonium carbonate, some phenol, skatol, trimethylamine, and the volatile fatty acids. The residue after distillation is treated in succession by ether and by alcohol.

The extraction with ether (B) separates the ptomaïnes and some fatty acids. The alcoholic extract (C) removes the remainder of the fatty acids, as well as the acid and neutral nitrogenized bodies, almost all of which are crystallizable. The insoluble residue is boiled with dilute hydrochloric acid, with exclusion of air, finally evaporated to dryness, and the residue again extracted with alcohol. This new alcoholic solution (D) can be divided by acetate and subacetate of lead into two principal portions.

By operating in this manner the complex products of putrefaction are readily separated into four portions.

In his more recent work, GAUTIER has employed the following method: The putrid liquids, after the removal of fats, are feebly acidified with very dilute sulphuric acid, then distilled in vacuo at a low temperature. The distillate contains ammonia, phenol, indol, and skatol. The syrupy residue, separated from any crystals which may have formed, is rendered alkaline with baryta, filtered, and extracted a great number of times with chloroform, in order to dissolve the bases. The solution is distilled at a low temperature, either in vacuo or in a current of carbonic acid. The contents of the retort, on being treated with water and tartaric acid, separate into a brown resin and a liquid portion. The latter is removed and treated with a dilute solution of potash, when it gives off the odor of carbylamine, which was discovered by GAUTIER in 1866, and which, according to CALMEL, is a constituent of the venom of toads. The alkali also sets free the bases, which are removed by extraction with ether, and the ether evaporated in a current of carbonic acid gas under slight pressure, then under a bell-jar over caustic potash. The bases may be separated by fractional precipitation with platinum chloride, or, if present in sufficient quantity, by distillation in vacuo.

Still later, GAUTIER has modified his method as follows: The alkaline putrid liquid is treated with oxalic acid (instead of sulphuric acid) to free acidulation and as long as the fatty acids continue to separate. The liquid is then warmed and distilled as long as a turbid fluid passes over. Pyrrol, skatol, phenol, indol, volatile fatty acids, and some of the ammonia pass over. The portion which remains in the retort is rendered alkaline with lime-water. The precipitate which forms, and which contains the greater part of the fixed fatty acids, is removed. The liquid portion, which is alkaline, is distilled to dryness, care being taken to receive the distillate in very dilute sulphuric acid. The bases and ammonia pass over. The distillate is neutralized (with sulphuric acid) and evaporated almost to dryness, then decanted from ammonium sulphate, which crystallizes. The mother-liquor is extracted with concentrated alcohol, which dissolves the sulphates of the ptomaïnes. After driving off the alcohol, the residue is rendered alkaline with caustic soda, and successively extracted with ether, petroleum ether, and chloroform.

The lime precipitate is dried and extracted with ether of thirty-six degrees, which removes any fixed bases that may be present.

REMARKS UPON THE METHODS. — The fundamental difference between the STAS-OTTO and the DRAGENDORFF methods consists in the fact that in the former the first extraction is made with a dilute solution of an organic acid (tartaric usually), while in the second a similar solution of a mineral acid (sulphuric) is employed. In their various modified forms any solvent may be used for separating the alkaloid from the other constituents of the original solution. Therefore, the question has been asked, Which is the more suitable acid for use in making the first solution? The answer to this question will also be the one to the question, Which is the better method of extracting ptomaïnes, the STAS-OTTO method or that of DRAGENDORFF? The Italian chemists GUARESCHI and MOSSO have attempted to answer this question experimentally, and the evidence which they have furnished is condemnatory of the method of DRAGENDORFF. They show that basic bodies are formed by the action of the dilute sulphuric acid upon albuminous substances. As this point is of vital importance to the investigator in this branch of chemical science, we will give a brief abstract of the work of GUARESCHI and MOSSO:

One kilogramme of fresh meat was treated with dilute sulphuric acid (in the proportion recommended in the DRAGENDORFF method) and alcohol. The dark solution after filtration was made alkaline with ammonium hydrate and extracted with ether. The ethereal solution gave on evaporation an oily substance which had the odor of extracts obtained from putrid fibrin. This substance, which was obtained in considerable quantity, was soluble in water and strongly alkaline in reaction. After neutralization with hydrochloric acid, its aqueous solutions gave the following alkaloidal tests:

(1) With platinum chloride, a yellowish-red precipitate, insoluble in water, alcohol, and ether, and apparently identical with the compound obtained from putrid fibrin with the same reagent.

(2) With gold chloride, yellow precipitate, then reduction to metallic gold.

(3) With phosphomolybdic acid, a heavy, yellow precipitate, forming a blue solution on the addition of ammonium hydrate.

(4) With phosphotungstic acid, a white precipitate.

(5) With MAYER's reagent, a heavy, whitish precipitate.

(6) With picric acid, white precipitate, instantly.

(7) With iodine in potassium iodide solution, a heavy kermes-red precipitate.

(8) With tannic acid, white precipitate.

(9) With mercuric chloride, white, amorphous precipitate.

(10) With MARMÉ's reagent, heavy precipitate.

(11) With potassium ferricyanide, no precipitate, but a cloudiness, with a formation of Prussian blue on the addition of ferric chloride.

The same quantity of this meat was also treated by the STAS-OTTO method. The alcoholic extract was evaporated on the water-bath and not in vacuo. The acid was neutralized with sodium bicarbonate. The ether extract gave on evaporation a faintly yellow residue, of not unpleasant odor and feebly alkaline reaction. After neutralization with hydrochloric acid, it was only slightly soluble in water. The pale-yellow filtrate gave no precipitate with Nos. 1, 2, 8, 9, and 10 of the above-mentioned reagents, but gave a slight turbidity with Nos. 3, 4, 5, 6, and 7, and with 11 formed Prussian blue.

GUARESCHI and MOSSO conclude from this and other experiments that the DRAGENDORFF method is not suitable for the extraction of ptomaïnes, and they recommend the employment of the STAS-OTTO method with these conditions: (1) no more acid should be added than is absolutely necessary to keep the reaction acid; (2) the heat used in evaporation should not be great, and it is better that evaporation should be made in vacuo. In this way, they say, no ptomaïne will be obtained from fresh tissue.

The same investigators extracted fresh flesh without the addition of any acid. Thirty kilogrammes of perfectly fresh meat were digested for two hours at from 50° to 60° with about one and one-half volumes of water. The fluids of the meat contained enough acid to give to the whole of this solution an acid reaction. It was evaporated to half its volume on the water-bath, filtered, and evaporated still further. The small residue was taken up with about four volumes of 96 per cent. alcohol. The reddish, alcoholic solution left on evaporation on the water-bath a brownish residue, which was dissolved in water and extracted with ether (A), then the solution was made alkaline with ammonium hydrate and again extracted with ether (B).

A gave on evaporation and cooling crystals of methyl-hydantoin, while the mother-liquor contained acetic acid.

B also yielded crystals of methyl-hydantoin, while the mother-liquor gave alkaloidal reactions with most of the general alkaloidal reagents, none with platinum chloride. Methyl-hydantoin does not give these reactions.

MARINO-ZUCO has made many comparative tests with these two methods. He ascertained that by treating fresh eggs, brain, liver, spleen, kidney, lungs, heart, and blood by either of the methods, he could obtain a substance which gave alkaloidal reactions, and which he demonstrated to be choline. His experiments led him to believe that choline did not exist pre-formed in these fresh tissues, but that it resulted from the action of the dilute acids upon lecithin. It was found most abundantly in those tissues which are rich in lecithin, such as the yolks of eggs, brain, liver, and blood; while only traces could be obtained from the whites of eggs, lungs, and heart. The method of DRAGENDORFF was found to furnish much larger quantities of choline than could be obtained by the STAS-OTTO method.

COPPOLA agrees with his countrymen, mentioned above, in condemning the method of DRAGENDORFF.

Enough has been said to show that results obtained by the STAS-OTTO method are much more reliable than those secured by the method of DRAGENDORFF. However, the former is not a perfect method, nor has a perfect one yet been devised. The principal difficulties met with in the STAS-OTTO method are as follows:

(1) In most instances the extraction of the base is very incomplete. (2) The degree to which the putrefactive alkaloid is removed by the solvent will depend very largely upon the nature of the other substances present. This fact in some cases aids and in others hinders the labors of the investigator. Thus, several ptomaïnes, which when pure are wholly insoluble in ether, may be removed, in part at least, from organic mixtures by this solvent by passing into the solution along with other substances, but if the attempt is made to purify one of these bases by repeated solution and extraction with ether, the result is a failure, because the more perfectly the alkaloid is freed from impurities, the less soluble it is in ether. This criticism, however, is equally applicable to the DRAGENDORFF method, and to all others in so far as extractions are made.

However, we may state that whenever it is applicable this method is the best now employed. By it the substances are submitted to the least chemical manipulation, and the results obtained are the most reliable. Many of the more complex putrefactive products are so easily decomposed or otherwise altered that the investigator should seek to isolate them by the simplest methods possible. If it can be done without the addition of any acid or without the application of heat, so much the better.

Especially is the modification of this method employed by MARINO-ZUCO, and already described, to be commended.

By his method, BRIEGER has discovered a considerable number of basic bodies and has given great impetus to the study of the chemistry of putrefaction. The method is capable of a great many modifications. As long ago as 1868, BERGMANN and SCHMIEDEBERG employed precipitation with metallic salts in order to obtain sepsine from putrid yeast. The method used by them was as follows: Putrid yeast was diffused through parchment paper; the diffusate was acidified with hydrochloric acid, and treated with mercuric chloride solution until a heavy cloudiness and, after some time, a slight precipitate formed. This was removed by filtration; the filtrate was rendered strongly alkaline with sodium carbonate, and then further treated with a solution of mercuric chloride as long as a precipitate formed. This precipitate was collected on a filter, washed, suspended in a little acidified water, and decomposed with hydrogen sulphide. The precipitate was removed, the free hydrochloric acid in the filtrate taken up with silver carbonate, and the excess of silver removed with hydrogen sulphide. The filtrate was evaporated to dryness; the residue dissolved in alcohol (a part remaining insoluble), and acidified with sulphuric acid, when a colorless or slightly yellow crystalline precipitate formed. The crystalline sepsine sulphate was purified by solution in water and precipitation with alcohol.

BRIEGER has obtained some of his bases by a much simplified modification of his complete method, which we have given in full. For instance, in obtaining neuridine, he treated the aqueous extract of the putrid material, after boiling and filtration, with mercuric chloride, collected the precipitate, decomposed it with hydrogen sulphide, evaporated the filtrate on the water-bath, and extracted the base from the residue with dilute alcohol.

By this method and its modifications **Brieger** has obtained many brilliant results, among which may be mentioned his discovery of mytilotoxine, typhotoxine, and tetanine. However, the method is not free from criticism. The great number of chemical manipulations to which the organic matter is subjected is liable to lead to the formation of some basic substances and to the destruction of others. One is justified in considering the isolated base as preexisting in the original material only when it produces symptoms identical with those caused by the substance from which it is extracted. There can be no doubt that by this method many ptomaïnes would be decomposed. With it **Ehrenberg** obtained from poisonous sausage only inert bases, and tyrotoxicon, the ptomaïne of poisonous cheese, is decomposed both by heat and the hydrogen sulphide employed. The origin of the ptomaïnes possessing a muscarine-like action discovered by **Brieger** has been questioned by **Gram**, who states that when the lactate of choline, an inert substance which is widely distributed both in plants and animals, is heated, it is converted into a poison with such an action.

CHAPTER IX.

METHODS OF ISOLATING THE BACTERIAL PROTEIDS.

HANKIN employed the following process in preparing his anthrax proteid :

"The cultures are made in 0.1 per cent. Liebig's extract of meat solution, to which some fibrin is added. The Liebig's extract is very difficult to sterilize, and must be heated for two or three hours in the steam sterilizer on two or three successive days. The fibrin must be added only after this has been done, and then the flask is re-sterilized by repeated heating to boiling-point, for a short time only on each occasion. If the fibrin were added at first it would be decomposed by the prolonged boiling. By the above method this only occurs to a slight degree, a mere trace of peptone being present in the sterilized culture-fluid. After sterilizing, this is inoculated with the blood of an animal dead of anthrax, and kept at the ordinary temperature. The anthrax forms a typical growth on the masses of fibrin, and samples of the liquid removed on successive days show a gradual increase in the strength of their biuret reaction. After about a week the liquid is filtered and the albumose extracted. The reason for not keeping the flask at a temperature of 37° is that the albumose is gradually decomposed into peptone by the anthrax ferment present, and this change takes place more rapidly at the higher temperature. For instance, I have found scarcely a trace of albumose in a culture which had been kept at 37° for a week, and which gave a strong biuret reaction. The albumose is separated from the culture-liquid thus prepared by saturation with ammonium sulphate. It is better to acidulate it slightly by adding a little acetic acid. The bulky precipitate of albumose which then appears is filtered off, and the salt separated from it by dialysis. An excess of thymol must be added at this stage to prevent putrefaction, or the dialysis can be carried on in a current of water which is warmed to from 45° to 50° C., at which temperature the growth of microörganisms is inhibited. After dialyzing for twenty-four hours or more the greater part of the salt will have vanished, and the albumose will be found in solution in a considerable quantity of water which will not have passed through the parchment. It is now necessary merely to concentrate the solution and precipitate the albumose by the addition of alcohol. In my earlier experiments this was accomplished by evaporating *in vacuo* at a temperature of 45° to 48°. When at length the liquid has been reduced to a few cubic centimetres it is poured into alcohol, and the precipitated albumose is filtered off, washed with the same reagent (alcohol), and dried.

"Evaporating *in vacuo* is a long and tedious process, and it requires a somewhat complicated apparatus. When it is used for pathogenic albumoses there is always a risk of the temperature employed destroying or diminishing their physiological properties. Further, if the albumose is allowed to evaporate to dryness, it may be difficult to make it pass into solution again. To avoid these difficulties I have designed a method of concentrating such solutions which is less objectionable. It depends on the principle that, if alcohol and water are placed on opposite sides of a membrane, the water rapidly dialyzes through to mix with the alcohol, while only traces of alcohol pass through to mix with the water. Consequently, if a watery solution of albumoses is dialyzed against alcohol, the solution diminishes in bulk and is rapidly concentrated, owing to the passage of the water through the membrane.

"My *modus operandi* is to place the dilute albumose solution in a parchment sausage skin which is immersed in a foot glass full of methylated spirit. The spirit can be changed after some hours if it is desired to prolong the process; but this is not usually necessary. In this way I have been able to bring 400 c.c. of albumose solution down to 100 c.c. in the course of a single night, at the ordinary temperature, without risk to the albumose or trouble to myself. The concentrated solution is then poured into absolute alcohol, which precipitates the albumose and removes any impurities that might be derived from the methylated spirit. This prolonged treatment with alcohol will tend to remove any free ptomaïnes or other substances soluble in alcohol. Peptones and salts present in the culture liquid remained for the most part in solution when the albumose was precipitated with $(NH_4)_2SO_4$. No soluble proteids (except traces of peptone) were present in the culture medium."

Ordinarily the bacterial proteids are isolated by precipitation with absolute alcohol, re-solution in water and reprecipitation with alcohol. However, as has been stated, TIZZONI and CATTANI find that strong alcohol destroys the activity of the poison of their tetanus germ. The method employed in obtaining the bacterial cellular proteids has already been given (see page 130).

CHAPTER X.

THE IMPORTANCE OF PTOMAÏNES TO THE TOXICOLOGIST.

THE presence in the cadaver of substances which give not only the general alkaloidal reactions but respond to some of the tests which have hitherto been considered characteristic of individual vegetable alkaloids, must be of the greatest importance to toxicologists. The possibility of mistaking putrefactive for vegetable alkaloids should always be borne in mind by the chemist in making his medico-legal investigations. On the other hand, as we have seen in preceding chapters, cases of poisoning by ptomaïnes sometimes terminate fatally, and in such instances the chemist should not be satisfied with determining the absence of mineral and vegetable poisons, but should strive to detect in the food or in the dead body positive evidence of the presence of the putrefactive alkaloid.

We will give a brief account of those cases in which putrefactive substances have been found to resemble in their reactions the vegetable alkaloids.

CONIINE-LIKE SUBSTANCES.—The most celebrated case in which a substance giving reactions similar to those of coniine has been found, was the Brandes-Krebs trial, which took place in Braunschweig in 1874. From the undecomposed parts of the body two chemists obtained, in addition to arsenic, an alkaloid which they pronounced coniine. This substance was referred to OTTO for further examination. OTTO reported that the substance was neither coniine nor nicotine, nor any vegetable alkaloid with which he was acquainted. OTTO converted the substance into an oxalate, dissolved it in alcohol, evaporated the alcohol, dissolved the residue in water, rendered this solution alkaline with potash, then extracted the base with petroleum ether. On evaporation of the petroleum ether the alkaloid appeared as a bright yellow oil, which had a strong, unpleasant odor, quite different, however, from that of coniine. It was strongly alkaline and had an intensely bitter taste. At ordinary temperature it was volatile. From its aqueous solution it was precipitated by the chlorides of gold, platinum, and mercury. In these reactions it resembled nicotine, from which it differed in the double refractive and crystalline character of its hydrochloride. With an ethereal solution of iodine this substance did not give the ROUSSIN test for nicotine, but instead of the long ruby-red crystals there appeared small, dark-green, needle-shaped crystals.

This substance was found to be highly poisonous. Seven centigrammes injected subcutaneously into a large frog produced instantaneous death, and forty-four milligrammes given to a pigeon caused a similar result. On account of its poisonous properties the jury of medical experts decided that the substance was a vegetable alkaloid. OTTO says that this decision astounded the chemists.

BROUARDEL and BOUTMY found in the body of a woman, who had died, after suffering, with ten other persons, from choleraic symptoms from eating of a stuffed goose, a base which gave the odor of coniine and the same reactions with gold chloride and iodine in potassium iodide, etc., as coniine. The same base was found in the remainder of the goose. But it did not give a red coloration with the vapor of hydrochloric acid, and it did not form butyric acid on oxidation, and although it was poisonous, it did not produce in frogs the symptoms of coniine poisoning.

SELMI repeatedly found coniine-like substances in decomposing animal tissue. By distilling an alcoholic extract from a cadaver, acidifying the distillate with hydrochloric acid, evaporating, treating the residue with barium hydrate and ether, and allowing the ether to evaporate spontaneously, he obtained a residue of volatile bases, the greater part of which consisted of trimethylamine. After removing the trimethylamine, the residue had the odor of the urine of the mouse. Later, SELMI obtained an unmistakable coniine odor from a chloroform extract of the viscera of a person who had been buried six months, and in another case ten months after burial. Two or three drops of an aqueous solution of the alkaline residue of the chloroform extract allowed to evaporate on a glass plate gave off such a penetrating odor that SELMI was compelled to withdraw from close proximity to the substance. The odor imparted to his hands in testing the substance with the general alkaloidal reagents remained for half an hour. This volatile base seemed to be formed by the spontaneous decomposition of other ptomaïnes.

An aqueous solution of a ptomaïne obtained by SELMI by extraction with ether according to the STAS-OTTO method from the undecomposed parts of a cadaver had no marked odor, but after having been kept for a long time in a sealed tube it not only gave off a marked coniine odor, but the vapor turned red litmus-paper blue. Again, the sulphate of a ptomaïne obtained from putrid egg-albumin, on standing formed in two layers, one of which was a golden-yellow liquid, which on being treated with barium hydrate gave off ammonia, and later, the odor of coniine. Since butyric and acetic acids were formed by the oxidation of this base, SELMI concluded that he had real coniine or methylconiine, and that it was formed by the oxidation of certain fixed ptomaïnes, or by the action of different amido bases on volatile fatty acids. Therefore SELMI believed in the spontaneous origin of coniine or closely allied bases in putrid matter, also in the existence of a "cadaveric coniine."

The substance which was found by SONNENSCHEIN in a criminal trial in East Prussia, and which was believed by that chemist to be the alkaloid of the water hemlock (cicuta virosa), is thought by OTTO, HUSEMANN, and others, to be a cadaveric coniine. OTTO says that the symptoms reported in the case were not those of either coniine or cicuta. SONNENSCHEIN obtained the base six weeks after the exhuming of the body, which had been buried three months. The base had the odor of coniine, the taste of tobacco, gave with potassium bichromate and sulphuric acid the odor of butyric acid, and behaved with reagents like coniine.

HUSEMANN states that at present it is very difficult, if not impossible, for the chemist to state with certainty that he has detected true coniine in the dead body. The symptoms and the post-mortem appearances must conform with those induced by the vegetable alkaloid. The analysis must be made before decomposition sets in, and the amount of the base found must be sufficient for physiological experiments to be made with it.

A NICOTINE-LIKE SUBSTANCE.—WOLCKENHAAR obtained from the decomposed intestines of a woman, who had been dead six weeks, by extraction with ether from an alkaline solution, a base which bore a close resemblance to nicotine. The base was fluid, at first yellow, but on being exposed to the air, brownish-yellow. It was strongly alkaline in reaction and gave off an odor resembling nicotine, but stronger, not ethereal, but benumbing and similar to that of fresh poppy-heads. It was soluble in all proportions in water, and the solutions, which did not become cloudy on the application of heat, did not taste bitter, but were slightly pungent. The peculiar odor did not disappear on saturating the base with oxalic acid. The hydrochloride was yellow, like varnish, had a strong odor, and became moist on exposure to the air. Under the microscope it showed no crystals, differing in this respect from nicotine hydrochloride. It differed from nicotine also in its reactions with potassio-bismuthic iodide, gold chloride, iodine solution, mercuric chloride, and platinum chloride. It also failed to give the ROUSSIN test for nicotine. Moreover, it could not be identified with trimethylamine, sparteine, mercurialine, lobeline, or other fluid and volatile bases.

The studies of RÖRSCH and FASSBENDER (page 28), of SCHWANERT (page 28), of LIEBERMANN (page 30), and of SELMI (page 31), have already been referred to in a preceding chapter.

STRYCHNINE-LIKE SUBSTANCES.—In a criminal prosecution at Verona, CIOTTA obtained from the exhumed, but only slightly decomposed body, an alkaloid which gave a crystalline precipitate with iodine in hydriodic acid, a red coloration with hydriodic acid, and a color test similar to that of strychnine with sulphuric acid and potassium bichromate, and with other oxidizing agents. This substance was strongly poisonous, but did not produce the tetanic convulsions which are characteristic of strychnine. CIOTTA pronounced this substance as probably identical with strychnine. Portions of the body were subsequently submitted to SELMI for his opinion. SELMI found that the substance which gave the color-reaction was not crystalline, and that there was only "the presumption of a bitter taste to it," while one part of strychnine in 40,000 parts of water is intensely bitter. SELMI also held that many ptomaïnes give reactions similar to strychnine with iodine in hydriodic acid and with hydriodic acid. He also held that its physiological properties were such that it could not be strychnine. This substance could hardly have been aspidospermine, which reacts with sulphuric acid and potassium bichromate similarly to strychnine, because quebracho bark, in which this alkaloid is found, was not at that time used as a medicine or known in Italy.

Ptomaïnes giving reactions similar to those of strychnine, and also causing tetanic spasms, have been found in Italy in decomposed corn-meal. SELMI obtained one of these substances, but found that it differed from strychnine inasmuch as it could not be extracted with ether.

LOMBROSO has named the poisonous substance found in decomposed corn-meal pellagroceine, but this is really a mixture of ptomaïnes, some of which produce narcosis and paralysis, and others produce the symptoms of nicotine poisoning instead of the spasms caused by strychnine.

A MORPHINE-LIKE SUBSTANCE.—In the Sonzogna trial, at Cremona, Italy, the experts seem to have confounded a ptomaïne with morphine. This substance was not removed from either alkaline or acid solutions with ether, but could be extracted with amylic alcohol. It reduces iodic acid, but in its other reactions, as well as in its physiological properties, it bore no resemblance to morphine. In frogs it arrested the heart in systole, which is said never to happen in poisoning with morphine. It failed to give both the ferric chloride and the Pellagri tests for morphine.

In the same body there was found a substance which was extracted from alkaline solutions with ether, and which gave, with hydrochloric acid and a few drops of sulphuric acid, on the application of heat, a reddish residue similar to that obtained by the same reagents with codeine, but in its other reactions it did not resemble this alkaloid.

ATROPINE-LIKE SUBSTANCES.—Many investigators have found products of putrefaction which in their mydriatic properties resemble atropine and hyoscyamine. To this class belongs the substance observed by ŽUELZER and SONNENSCHEIN. It was removed from alkaline solutions by ether, and formed microscopic crystals, an aqueous solution of which, when applied to the conjunctiva, produced a mydriatic effect, and, when administered internally, increased the action of the heart and arrested the movements of the intestines. Moreover, with certain alkaloidal reagents, such as platinum chloride, it resembled atropine. But when heated with sulphuric acid and oxidizing agents it did not give the odor of blossoms (REUSS's test). However, SELMI found ptomatropines which with sulphuric acid and oxidizing agents did give the blossom odor as distinctly as the vegetable atropine. These putrefactive bases also developed this odor spontaneously after standing for two or three days, and this does not happen with atropine. The odor was produced with the ptomatropines by nitric and sulphuric acids, both in the cold and on the application of heat, while these acids in the cold do not produce the odor with atropine.

DIGITALINE-LIKE SUBSTANCES.—Elsewhere we have referred to the discovery of a ptomaïne belonging to this class by **Rörsch** and **Fassbender** (see page 28). **Trottarelli** obtained a similar substance from the brain of a man in whose abdominal viscera he could find no poison. The sulphate of this base gave on evaporation an aromatic-smelling and astringent-ta-ting residue. It became purple with sulphuric acid alone, and dark red with hydrochloric and sulphuric acids. On frogs this ptomaïne showed no toxic effect.

A VERATRINE-LIKE SUBSTANCE.—**Brouardel** and **Boutmy** obtained from a corpse which had lain in water for eighteen months, and a large portion of which had changed into adipocere, a ptomaïne resembling veratrine. It was removed from alkaline solutions by ether. On being heated with sulphuric acid it became violet. With a mixture of sulphuric acid and barium peroxide it became, in the cold, brick-red; and, on being heated, violet. With boiling hydrochloric acid it took on a cherry-red coloration. However, it differed from veratrine, inasmuch as it reduced ferric salts instantly, and when injected into frogs subcutaneously it did not induce in them the spasmodic muscular contractions characteristic of veratrine.

Bechamp obtained by the **Stas-Otto** method from the products of the pancreatic digestion of fibrin an alkaloid body which gave with sulphuric acid a beautiful carmine-red, similar to that given with veratrine. By digesting this substance with gastric juice, and again extracting, he obtained a body which behaved with sulphuric acid similar to curarine.

A DELPHININE-LIKE SUBSTANCE.—In 1870, General Gibbone, an Italian of prominence, died suddenly. His servant was accused of having poisoned him. Two chemists of some reputation reported the presence of delphinine in the viscera. It seemed somewhat improbable that the servant should know anything of so rare a substance, or that he should have been able to obtain it. However, two or more varieties of staphisagria grow in Southern Italy, and it was possible that the servant had used some preparation made by himself from the plant. The supposed alkaloid was given to SELMI, of Bologna, for further study. It was removed from alkaline solutions by ether. When heated with phosphoric acid it became red, and when brought in contact with concentrated sulphuric acid, reddish-brown. In these tests the substance resembled delphinine, but with sulphuric acid and bromine water, also with FRÖHDE'S reagent, the colorations characteristic of the vegetable alkaloid failed to appear. Moreover, SELMI showed that delphinine gave the following reactions, to which the suspected substance did not respond: (1) Delphinine dissolved in ether, and treated with a freshly prepared ethereal solution of platinic chloride, gives a white, flocculent precipitate which is insoluble in an equal volume of absolute alcohol. (2) Delphinine gives precipitates with auro-sodium hyposulphite, and with a sulphuric acid solution of cupro-sodium hyposulphite, the latter precipitate being soluble in an excess of the reagent.

Finally, CIACCIA and VELLA showed that while delphinine arrests the heart of the frog in diastole, the suspected substance arrests it in systole.

A COLCHICINE-LIKE SUBSTANCE.—BAUMERT found in a suspected case of poisoning, twenty-two months after death, a substance which gave many of the reactions for colchicine. It was extracted from acid solutions with ether, to which it imparted a yellow color. On evaporation of the ether a yellow, amorphous substance remained, and this dissolved in warm water with yellow coloration. It could be extracted from acid solutions also by chloroform, benzol, and amylic alcohol, but not by petroleum ether. It was removed with much more difficulty from alkaline solutions.

All the extracts were yellow, and left on evaporation a feebly alkaline, markedly bitter, sharp-tasting, amorphous, yellow residue, which dissolved in water and dilute acids incompletely, forming a resin. When this resin was dissolved in dilute sodium hydrate, and the solution rendered acid by sulphuric acid, the same reactions were obtained as with the original extract.

With phosphomolybdic acid, phosphotungstic acid, potassio-bismuthic iodide, potassio-mercuric iodide, iodine in potassium iodide, tannic acid and gold chloride, this substance gave the same reactions which were obtained by parallel experiments with genuine colchicine; thus, the tannic acid precipitates were both soluble in alcohol, and the precipitates with phosphomolybdic acid in both cases became blue on the addition of ammonium hydrate.

Concentrated sulphuric and dilute nitric and hydrochloric acids dissolved the supposed colchicine with yellow coloration. Strong nitric acid (1.4 sp. gr.) colored the substance dirty red, scarcely to be called a violet. When the substance was purified as much as possible, this color became a beautiful carmine-red. The addition of water changed the red into yellow, and caustic soda produced a dark, dirty orange.

In general, in the above-mentioned reactions, the putrefactive product agreed with the real colchicine, but the former gave precipitates with picric acid and platinum chloride, while the latter gives no precipitates with these reagents.

In 1886, ZEISEL proposed the following test for colchicine: When a hydrochloric acid solution of the alkaloid is boiled with ferric chloride, it becomes green, sometimes dark-green and cloudy. Now, if the fluid be agitated with chloroform, the chloroform will sink, taking up the coloring matter, and appearing brownish, granite-red or dark, and the supernatant fluid clears up without becoming wholly colorless.

BAUMERT applied this test to both colchicine and the putrefactive product. To from two to five cubic centimetres of the suspected solution in a test-tube, he added from five to ten drops of strong hydrochloric acid and from four to six drops of a ten per cent. solution of ferric chloride, then heated the mixture directly over a small flame until it was evaporated to half its volume or less. In the presence of one milligramme of colchicine the originally bright-yellow solution became gradually olive-green, and, on further concentration, dark-green and cloudy. Then, on shaking the fluid with chloroform, admitting as much air as possible, the chloroform subsided, having a ruby-red color if as much as two milligrammes of colchicine were present, and a bright yellow if only one milligramme, and the supernatant fluid became of a beautiful olive-green. When ether, petroleum ether, benzol, carbon disulphide, or amylic alcohol was substituted for the chloroform, the coloration did not appear. From this BAUMERT infers that the red coloring matter is either only soluble in chloroform, or that it is not formed until the chloroform is added.

BAUMERT found this test of great value in deciding whether or not the substance which he found was colchicine. The putrefactive product did not respond to the test.

Some of this substance was sent to BRIEGER, who decided that it was not a base, but a peptone-like substance. It was also found to be inert physiologically.

Before these investigations were made by BAUMERT, LIEBERMANN had found the same or a similar colchicine-like substance in the cadaver. His description differed from that of BAUMERT only in regard to the taste of the substance, LIEBERMANN having failed to observe any marked taste in the substance which he found, while, as has been stated, BAUMERT reported a distinctly bitter taste.

A colchicine-like substance has been found in beer, and it has been suggested that it was this which the above-mentioned toxicologists found in the bodies which they examined, but LIEBERMANN states that the man whose body he examined had been a total abstainer from beer.

TAMBA compared the reactions of ptomaïnes obtained from putrid sausage with similar reactions of various alkaloids, and then ascertained the effect upon the alkaloidal reactions by mixing the alkaloids with the ptomaïnes. His results are as follows:

MORPHINE.—Ptomaïnes are colored yellow with nitric acid; reddish-yellow with concentrated sulphuric acid;

blue, violet, then green with FRÖHDE's reagent; yellow when evaporated with concentrated sulphuric acid, then treated with hydrochloric acid and decomposed with sodium bicarbonate. The ptomaïnes reduce ferric chloride, but not iodic acid. With sugar and concentrated sulphuric acid, they give a yellow coloration.

Mixtures of the ptomaïnes and morphine give absolutely characteristic reactions for morphine with sugar and sulphuric acid, the violet coloration appearing distinctly; and by evaporation on the water-bath with sulphuric acid, addition of hydrochloric acid and decomposition with sodium bicarbonate, the violet color appearing. Iodic acid is reduced by morphine in the presence of ptomaïnes, only when the ptomaïnes are present in minute quantity.

The other reactions for morphine are not applicable in the presence of ptomaïnes.

STRYCHNINE.—The characteristic color reaction for this alkaloid, with potassium bichromate and sulphuric acid, is not affected by the presence of ptomaïnes.[1]

BRUCINE.—The nitric acid reaction for brucine is not affected by ptomaïnes. On the other hand, the reaction with sulphuric and nitric acids, in which a red coloration is obtained, is scarcely visible in the presence of ptomaïnes. The action of mercuric nitrate and heat on brucine, by which a violet coloration is produced, is not destroyed by the presence of ptomaïnes.

VERATRINE.—The characteristic coloration of veratrine by concentrated sulphuric acid is not influenced by ptomaïnes. The same is true of the cherry-red coloration with concentrated hydrochloric acid. On the contrary, the action of sugar and sulphuric acid on veratrine is without result in the presence of ptomaïnes.

ATROPINE.—The deep violet coloration produced by fuming nitric acid, subsequent concentration, and the addition of alcoholic potassium hydrate, is not affected by the presence of ptomaïnes. On the other hand, the characteristic odor produced by the action of sulphuric acid and heat on atropine is scarcely recognizable when ptomaïnes are present.

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[1] In contradiction to this, see page 178.

NARCEINE.—The blood-red color produced by concentrated sulphuric acid fails in the presence of ptomaïnes.

COLCHICINE.—Fuming nitric acid colors the ptomaïnes reddish-yellow, but the violet coloration of colchicine with nitric acid appears in well-defined form, even in the presence of ptomaïnes. The other reactions for colchicine are valueless when ptomaïnes are present.

CODEINE.—The blue coloration of codeine with concentrated sulphuric acid holds good when ptomaïnes are present. The same is true of the reaction with sulphuric acid, heat, and the subsequent addition of nitric acid. FRÖHDE'S reagent fails with codeine when mixed with ptomaïnes, inasmuch as the bluish coloration rapidly passes into a brown.

ACONITINE.—Phosphoric acid and concentrated sulphuric acid are without reaction on the alkaloid when mixed with ptomaïnes.

PICROTOXINE.—The reducing action of picrotoxine on alkaline copper sulphate solution is seriously affected by the presence of ptomaïnes. The same is true of other tests for this poison.

DELPHININE.—The reaction of delphinine with sulphuric acid and bromine water, as well as the one with FRÖHDE'S reagent, is so much influenced by the presence of ptomaïnes that the alkaloid cannot be recognized.

These results are to be accepted with caution, as it is not reasonable to suppose that all ptomaïnes will affect the test for the vegetable alkaloids in the same manner or to the same degree. Moreover, there is no proof that Tamba worked with pure ptomaïnes.

Tamba has also proposed to separate vegetable from putrefactive alkaloids by adding to ethereal solutions of mixtures an equal volume of a saturated ethereal solution of oxalic acid, and allowing to stand, when the oxalates of the vegetable alkaloids will separate in crystalline form, and the oxalates of the ptomaïnes will remain in solution. In other words, the oxalates of the vegetable alkaloids are insoluble in ether, while the oxalates of the putrefactive alkaloids are soluble in ether. But, in contradiction to this, Bocklisch states that the oxalate of cadaverine is insoluble in ether.

The most important work which the toxicologist is called upon to do at present is to isolate and identify beyond all question the bacterial poisons. This work has become important on account of the frequent occurrence of poisoning from articles of infected food.

CHAPTER XI.

CHEMISTRY OF THE PTOMAÏNES.

THE basic substances described in the following pages are arranged, so far as possible, in the regular natural order. An inspection of the list of these bases will show the remarkable fact of the predominancy of the amine type. Almost two-thirds of the known ptomaïnes contain only C, H, and N, and represent simple ammonia substitution compounds. Of the oxygenated bases, all of those whose constitution is known possess the trimethylamine molecule as their basic constituent, and it is quite probable that most, if not all, of the remaining ptomaïnes will be found to possess the same or a similar basic nucleus.

It will be seen, furthermore, that a very large number of the ptomaïnes described possess little or no toxic action, and are, therefore, physiologically inert. It would seem, as BRIEGER has already pointed out, that a certain quantity of oxygen is necessary to the formation of poisonous bases. A free supply of oxygen, on the other hand, invariably yields non-toxic ptomaïnes. The poisonous bases begin to appear on about the seventh day of putrefaction, and in turn disappear if this is allowed to go on for a considerable period of time.

METHYLAMINE, $CH_3.NH_2$.—This is the simplest organic base that is formed in the process of putrefaction. It is ammonia in which one atom of hydrogen has been replaced by the methyl radical. It occurs in herring-brine (TOLLENS, 1866; BOCKLISCH, 1885); in decomposing herring, twelve days in spring (BOCKLISCH); in pike, six days in summer (BOCKLISCH); in haddock, two months at a low temperature (BOCKLISCH); in the fermentation of choline chloride (HASEBROEK). BRIEGER has shown it to be present in cultures of comma bacillus on beef-broth which were kept for six weeks at 37°–38°. EHRENBERG reported its possible presence in poisonous sausage, and obtained it by growing a bacillus from this source on intestines (1887). In BRIEGER's method, methylamine is found both in the mercuric chloride precipitate and filtrate. The mercury double salt is readily soluble in water, and can thus be separated from any accompanying cadaverine or putrescine. Methylamine is an inflammable gas of strong ammoniacal odor, and burning with a yellow flame. It is readily soluble in water, and its solutions give reactions similar to those of ammonia. Its salts are, as a rule, also soluble in both water and alcohol.

The HYDROCHLORIDE, $CH_3NH_2.HCl$, crystallizes in large deliquescent plates. On being heated with alkali, it gives off the odor of methylamine.

The PLATINOCHLORIDE, $(CH_3.NH_2.HCl)_2PtCl_4$ ($Pt = 41.31$ per cent.),[1] yields hexagonal plates which usually occur heaped up in several layers. It is soluble in about fifty parts of water at ordinary temperature, and can be readily recrystallized from hot water. It is insoluble in absolute alcohol and in ether.

The AUROCHLORIDE, $CH_3.NH_2.HCl.AuCl_3 + H_2O$, forms prisms, which are readily soluble in water. There is also a readily soluble picrate.

Methylamine does not possess any toxic action, even when given in fairly large doses. This physiological indifference is shared by nearly all the monamines and diamines that have been obtained among the products of putrefaction.

DIMETHYLAMINE, $(CH_3)_2.NH$, has been found in putrefying gelatin, ten days at 35° (BRIEGER, 1885); in yeast decomposing in covered vessels for four weeks during summer (BRIEGER); in decomposing perch, six days in summer (BOCKLISCH); and in herring-brine (BOCKLISCH, 1886). It has been found in poisonous sausage, and in cultures of a bacillus obtained from this source, on liver and intestines (EHRENBERG, 1887). It is also formed, together with trimethylamine, when neuridine hydrochloride is distilled with sodium hydrate (BRIEGER, I., 23). It occurs in the mercuric chloride precipitate as well as filtrate. From cadaverine it can be separated by platinum chloride, since cadaverine platinochloride is difficultly soluble in cold water, and recrystallizes from hot water, whereas the dimethylamine double salt remains in the mother-liquor. In like manner it can be separated from neuridine. From choline it can be isolated by recrystallizing the mercuric chloride precipitate from hot water.

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[1] The percentages given in the following pages are calculated from $Au = 196.64$ (Krüss), $Pt = 194.46$ (Seubert), $Cl = 35.37$, $O = 15.96$.

The free base is a gas at ordinary temperature, but can be condensed to a liquid which boils at 8°–9°. The hydrochloride, $(CH_3)_2.NH.HCl$, crystallizes in needles, which deliquesce on exposure to air and are soluble in absolute alcohol (BRIEGER, I , 56). It is insoluble in absolute alcohol (BOCKLISCH) but soluble in chloroform (BEHREND), and can then be separated from methylamine hydrochloride, which is insoluble in chloroform.

The PLATINOCHLORIDE, $[(CH_3)_2.NH.HCl]_2PtCl_4$, (Pt = 39.00 per cent.), crystallizes in long needles, which are easily soluble in hot water, less soluble in cold water. Sometimes it forms orange-yellow plates or prisms, or else small needles.

The AUROCHLORIDE, $(CH_3)_2.NH.HCl.AuCl_3$, forms needles (BOCKLISCH), or large yellow monoclinic plates (HJORTDAHL), which are insoluble in absolute alcohol.

TRIMETHYLAMINE, $C_3H_9N = (CH_3)_3N$, has been known for a long time to occur in animal and vegetable tissues. DESSAIGNES showed its presence in leaves of Chenopodium (1851), in the blood of calves (1857), and later in human urine. It has been obtained from ergot (Secale cornutum) by WALZ (1852) and BRIEGER (1886); from herring-brine by WERTHEIM, WINKLES, TOLLENS, and BOCKLISCH. In these substances, with the exception of herring-brine, it probably does not exist pre-formed, but is rather a product of the method employed for its isolation. In fact, BRIEGER has shown that it does not exist in ergot, but is formed at the expense of the choline present, which, on distillation with potash, decomposes and yields trimethylamine and glycol. Thus:

$$C_2H_4OH.N(CH_3)_3.OH = N(CH_3)_3 + C_2H_4(OH)_2.$$

It is also formed when betaine and neuridine are distilled with potash. It may have a similar origin in most of the other cases, since choline is now known to be widely disseminated in plants and animals, either as such or as a constituent of the more complex lecithin. Trimethylamine has been found in the putrefaction of yeast (HESSE, 1857; MÜLLER, 1858); in cheese after six weeks in midsummer (BRIEGER); in human liver and spleen after from two to seven days (BRIEGER); in perch after six days in midsummer (BOCKLISCH); in mussel (Mytilus edulis) after sixteen days (BRIEGER); in putrefying brains after from one to two months, and in fresh brains (GUARESCHI and MOSSO); in cultures of the Streptococcus pyogenes on beef-broth, bouillon, meat extract, and blood-serum, and from cultures of the comma bacillus (BRIEGER). It has also been found in cod-liver oil. EHRENBERG (1887) reports its presence in considerable quantity in poisonous sausage, and in cultures of a bacillus, isolated from this, grown on liver, intestines, and meat bouillon.

Trimethylamine is found both in the mercuric chloride precipitate and filtrate. It remains in the mother-liquor from which cadaverine, neuridine, and dimethylamine platinochlorides have crystallized. If an aqueous solution of mercuric chloride is used as the precipitant, the trimethylamine will be found almost entirely in the filtrate, from which it can be obtained after removal of the mercury by evaporating the filtrate to dryness, extracting with alcohol, and treating the solution thus obtained with alcoholic platinum chloride.

The free base is a liquid possessing a strong, fish-like odor. Its boiling-point is 9.3°. It is strongly alkaline in reaction and freely soluble in water.

The HYDROCHLORIDE, $(CH_3)_3N.HCl$, is deliquescent and freely soluble in water and alcohol. Heated to 285° it decomposes. With alkalies it gives off the odor of the free base.

The PLATINOCHLORIDE, $[(CH_3)_3N.HCl]_2PtCl_4$ (Pt = 36.92 per cent.), is soluble in hot water, from which, on cooling, it recrystallizes in orange-red octahedra or needles, which do not lose water when heated at 100°–110° (BOCKLISCH).

The AUROCHLORIDE, $(CH_3)_3N.HCl.AuCl_3$ (Au = 49.39 per cent.), is easily soluble, and hence can be separated from choline aurochloride, which is difficultly soluble. Similarly, this base can be separated from ammonia by the use of gold chloride.

Trimethylamine is not a strong poison, since very large doses of it must be given in order to bring out any physiological disturbances.

ETHYLAMINE, $C_2H_5.NH_2$, is formed in putrefying yeast (HESSE, 1857); in wheat flour (SULLIVAN, 1858); and also in the distillation of beet-sugar residues.

It is a strongly ammoniacal liquid boiling at 18.7°, and is miscible with water in every proportion. Like the other amines, it is combustible. It possesses strong basic properties, and is capable of expelling ammonia from its salts in a manner analogous to the action of the fixed alkalies.

The HYDROCHLORIDE, $C_2H_5.NH_2.HCl$, forms deliquescent plates, which melt at 76°–80°. It is readily soluble in water and alcohol.

The PLATINOCHLORIDE, $(C_2H_5.NH_2:HCl)_2PtCl_4$, forms orange-yellow rhombohedra (WELTZIEN), or hexagonal-rhombohedral crystals (TOPSOË).

The AUROCHLORIDE, $C_2H_5.NH_2.HCl.AuCl_3$, forms gold-yellow monoclinic prisms, readily soluble in water.

With picric acid it forms short brown prisms, not very soluble in water.

DIETHYLAMINE, $C_4H_{11}N = (C_2H_5)_2NH$, has been obtained by BOCKLISCH from pike which were allowed to putrefy for six days in summer; and by growing a bacillus obtained from poisonous sausage on intestines and on meat bouillon (EHRENBERG, 1887).

It is an inflammable liquid which boils at 57.5°, possesses strong basic properties, and is soluble in water.

The HYDROCHLORIDE, $(C_2H_5)_2NH.HCl$, crystallizes in needles (BOCKLISCH); in long needles and prisms from absolute alcohol; in plates from ether-alcohol. These are not deliquescent and are easily soluble in water and in chloroform; rather difficultly in absolute alcohol. Heated with sodium hydrate it gives off alkaline vapors. From an alcoholic solution it is precipitated by addition of alcoholic mercuric chloride. The mercury double salt is difficultly soluble in hot water, from which it recrystallizes on cooling.

The PLATINOCHLORIDE, $[(C_2H_5)_2.NH.HCl]_2PtCl_4$, crystallizes in orange-yellow monoclinic crystals, which are easily soluble in water.

The AUROCHLORIDE, $(C_2H_5)_2.NH.HCl.AuCl_3$ ($Au =$ 47.71 per cent.), forms trimetric crystals (TOPSOË), which are difficultly soluble (BOCKLISCH). It melts at about 165°.

With picric acid it forms an easily soluble picrate (LEA).

TRIETHYLAMINE, $C_6H_{15}N = (C_2H_5)_3N$, was obtained by BRIEGER (1885) from haddock which were exposed for five days in an open vessel during summer. He obtained it by distilling with potash, after removal of platinum by hydrogen sulphide, the mother liquor from which neuridine, the base $C_2H_8N_2$, muscarine, and gadinine had successively crystallized (see Gadinine). It has also been found by BOCKLISCH (1886) in putrid pike, and by EHRENBERG (1887). The latter obtained it from cultures of a bacillus, found in poisonous sausage, and grown on meat bouillon.

The free base is oily in character and possesses an ammoniacal odor. It is but slightly soluble in water, and boils at 89°–89.5°.

The PLATINOCHLORIDE, $[(C_2H_5)_3N.HCl]_2PtCl_4$ ($Pt =$ 31.84 per cent.), crystallizes in needles which are readily soluble in water.

With mercuric chloride the aqueous solution gives no precipitate.

With picric acid it yields yellow needles which are but slightly soluble in cold water.

PROPYLAMINE, $C_3H_7.NH_2$, is isomeric with trimethylamine, and can therefore be easily confounded with that base. There are two propylamines possible represented by the formulæ $CH_3.CH_2.CH_2.NH_2$ and $(CH_3)_2.CH.NH_2$. The former, or the normal compound, boils at 47°–48°, whilst the latter, or iso-propylamine, boils at 31.5°. Both are liquids possessing an ammoniacal, fish-like odor. They form crystalline salts; the hydrochlorides melt respectively at 155°–158°, and at 139.5°.

Iso-propylamine (?) has been found among the distillation products of the vinasse of beet-root molasses. Propylamine has been obtained by BRIEGER (1887) from cultures of the bacteria of human feces on gelatin. SCHWANERT has isolated from the organs of a cadaver a basic substance which was said to possess an odor similar to propylamine.

BUTYLAMINE, $C_4H_{11}N$, was obtained by GAUTIER and MOURGUES (1888) in cod-liver oil. It forms a colorless, mobile, alkaline liquid, the boiling-point of which they found to be 86° at 760 mm. It absorbs carbonic acid from the air and readily forms salts. The platinochloride forms golden-yellow plates which are quite soluble.

In animals it produces an increase in the function of the skin and kidneys, and in large doses fatigue, stupor, and vomiting.

ISO-AMYLAMINE, $C_5H_{13}N = (CH_3)_2.CH.CH_2.CH_2.NH_2$, has been obtained by LIMPRICHT in the distillation of horn with potash; it also occurs in the putrefaction of yeast (MÜLLER, HESSE, 1857); and in cod-liver oil (GAUTIER and MOURGUES, 1888), where it constitutes nearly one-third of the bases present.

It is a colorless, strongly alkaline liquid, possessing an odor which is not disagreeable. At the ordinary pressure it boils at 97°–98°.

The hydrochloride forms deliquescent crystals, which have a bitter, disagreeable taste. The platinochloride crystallizes in golden-yellow slender plates, which are very soluble in boiling water. The base is, according to GAUTIER and MOURGUES, identical with that obtained by treating iso-amylcarbimide with potash.

It is a very active poison, producing rigor, convulsions, and death. Four milligrammes produces death in a greenfinch in three minutes.

CAPROYLAMINE (HEXYLAMINE), $C_6H_{15}N$, has been found to occur by HESSE (1857) in the putrefaction of yeast. HAGER isolated from some putrid material what he thought to be a mixture of amylamine and caproylamine, and named it septicine.

Hexylamine was found, in small quantity, in cod-liver oil by GAUTIER and MOURGUES, and according to these authors it resembles amylamine in its action, but is less toxic.

TETANOTOXINE, $C_5H_{11}N$, (?) was obtained by BRIEGER (1886) as one of the products of the growth of the tetanus microbe on beef-broth or on brain-broth. It has also been obtained by KITASATO and WEYL (1890) from pure cultures of the tetanus bacillus, kept eight days at 36°. For its isolation see Tetanine, and *Ber.* **19**, 3120. It is tetanizing in its action, produces first tremor, then paralysis and violent convulsions. It forms an easily soluble gold double salt which melts at 130°. The platinochloride is difficultly soluble, and decomposes at 240°. The hydrochloride is crystalline, and is readily soluble in alcohol and in water. It melts at about 205°. From warm alcohol it crystallizes in flat, pointed plates.

SPASMOTOXINE, a base of as yet unknown composition, produces in animals violent clonic and tonic convulsions. It was obtained by BRIEGER (1887) from cultures of the tetanus germ on beef-broth.

Another *toxine* was obtained by BRIEGER (1887) in cultures of the tetanus microbe which produced complete tetanus, salivation, and tear-secretion. In its composition it is probably a diamine. The platinochloride forms plates which begin to decompose at 240°. The hydrochloride is very deliquescent. Gold chloride and picric acid form very soluble compounds. Besides these three bases he isolated another toxic substance, tetanine, and a base (see under Tetanine).

DIHYDROLUTIDINE, $C_7H_{11}N$, was found in cod-liver oil by GAUTIER and MOURGUES (1888). It is the first known hydrolutidine. It is a colorless, somewhat oily, very alkaline and caustic liquid, the odor of which is sharp, but somewhat agreeable when dilute. It absorbs carbonic acid from the air, darkens and thickens; is feebly soluble in water, and boils at 199° at 760 mm. pressure. The salts are bitter to the taste.

The hydrochloride crystallizes in a confused mass of needles or in plates. The nitrate reduces silver nitrate—a property of all hydropyridine bases (HOFMANN). The sulphate forms fine stellate deliquescent needles.

The platinochloride is readily precipitated from concentrated solutions as a canary-yellow precipitate. From warm solutions it crystallizes in lozenge-shaped plates which are often imbricated. On boiling with water it loses hydrochloric acid and forms $(C_7H_{11}NCl)_2PtCl_2$, which possesses a lighter color, is more soluble than the normal salt, and crystallizes confusedly.

The aurochloride crystallizes in needles which form fan or lozenge-shaped masses. It is scarcely altered even in hot water.

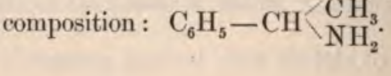
The IODOMETHYLATE, $C_7H_{11}N.CH_3I$, is obtained by mixing, in the cold, the base and methyl iodide. The colorless compound thus obtained is soluble in water and in alcohol, and possesses a disagreeable, somewhat nauseating odor. Treated with potash it yields a colorless, aromatic, very alkaline oil.

The base on oxidation with boiling potassium permanganate yields an acid, $C_7H_7NO_2$, and from this fact the discoverers conclude that the base is a dihydro-dimethyl-pyridine, $C_5H_4(CH_3)_2NH$.

Physiological Action.—It is moderately poisonous. In small doses it diminishes the general sensibility; in larger doses it produces trembling, especially of the head; profound depression alternating with periods of extreme excitement; paralysis of the posterior limbs, and death.

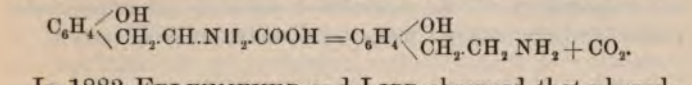
A BASE, $C_8H_{11}N$, isomeric, but not identical, with aldehyde-collidine, was obtained by **NENCKI** as early as 1876, by allowing a mixture of 200 grammes of pancreas and 600 grammes of gelatin in ten litres of water to putrefy for five days at 40°. The method used by **NENCKI** for its isolation is as follows: The fluid mass was distilled with sulphuric acid, to drive off the volatile acids, then rendered alkaline with barium hydrate, and again distilled. The distillate was received in dilute hydrochloric acid, and on evaporation gave a crystalline residue of ammonium chloride, and of a salt which formed in long rhombic plates. The latter were separated from the ammonium salt by absolute alcohol. The free base was obtained from the salt by treating it with sodium hydrate, and extracting the solution with ether.

This compound, as already stated, is isomeric with collidine, and also with O. DE CONINCK's base, with which it is possibly identical. The latter, however, will be described separately.

The free base is oily in character, and possesses a peculiar, not unpleasant odor. It readily absorbs carbonic acid gas from the air, forming after a time a lamellar, crystalline mass of the carbonate. The salt of this base on heating gives off an oil which burns with a smoky flame, and possesses an odor similar to that of xylol or cumol. **NENCKI** was therefore at first of the opinion that the ptomaïne was an aromatic base, probably an isophenyl-ethylamine of the following composition:  $C_6H_5—CH\begin{cases}CH_3\\NH_2\end{cases}$. He supposed that it was formed from the putrefaction of tyrosin, according to the following equation :

$$C_9H_{11}NO_3 = C_8H_{11}N + CO_2 + O.$$

We know that tyrosin does split up, on being heated to 270°, into carbonic acid and oxyphenyl-ethylamine, thus :



$$C_6H_4\Big\langle\begin{array}{l}OH\\CH_2.CH.NH_2.COOH\end{array} = C_6H_4\Big\langle\begin{array}{l}OH\\CH_2.CH_2\ NH_2\end{array} + CO_2.$$

In 1883 Erlenmeyer and Lipp observed that phenyl-α-amido-propionic acid (phenyl-alanine), on dry distillation, decomposed with the formation, among other products, of a base having the composition $C_8H_{11}N$. This base was found to be identical with phenyl-ethylamine, $C_6H_5.CH_2.CH_2.NH_2$, and in its properties and composition it resembles Nencki's base. Recently (1889), Nencki has taken up a similar view in regard to the nature of this base, and now regards it as possessing the formula just given—that it is phenyl-ethylamine. He regards phenyl-amido-propionic acid—one of the three aromatic nuclei contained in the albumin molecule—as the source of this base. From the fact that phenyl-α-amido-propionic acid is a well-known putrefactive product, it would seem that Nencki's base may arise either from the putrefactive decomposition of that acid, or from the splitting up of the acid as a consequence of the method employed in isolating the base. The latter would seem to be the most probable explanation of the genesis of this base, inasmuch as Brieger, by using his method for the isolation of ptomaïnes, has not been able to obtain it from putrid gelatin.

The Platinochloride, $(C_8H_{11}N.HCl)_2PtCl_4$ (Pt = 29.89 per cent.), is readily soluble in hot, and but slightly soluble in cold water, and can be, therefore, recrystallized from water. It forms beautiful flat needles. On dry heating it gives off an oil which possesses an odor resembling very much that of xylol or cumol, and burns with a smoky flame. This distinguishes Nencki's base from collidine, since the platinochloride of the latter does not show this behavior.

Nencki also obtained from putrid gelatin, under certain ill-defined conditions, especially when no glycocoll was present, a basic product which gave, with sulphuric acid, large lamellar crystals. The free base forms a thick colorless syrup, possessing a nauseous, bitter taste. It did not become crystalline even after standing some time. Unlike the base $C_8H_{11}N$, it is not volatile, and is, therefore, obtained on evaporation of the acidulated solution after previous removal of the volatile bases by distillation with baryta.

A BASE, $C_8H_{11}N$, isomer of collidine and of the preceding base, with which it is possibly identical, was obtained by O. DE CONINCK (1888) in the later stages of putrefaction of sea-polyps (*poulpes marins*). It forms a yellowish, rather mobile liquid, possessing a strong benumbing (*vireuse*) odor, and is but slightly soluble in water. It is soluble in methyl and ethyl alcohol, ether and acetone. Its density is 0.9865. When dried over potash it boils at 202° without undergoing decomposition. On exposure to the air it becomes brown, hydrates rapidly, and the boiling-point is then lowered. It has not been noticed to absorb carbonic acid from the air. It resembles some of the bases obtained from Dippel's oil. The salts are in general less stable than those of the pyridine bases, and in this respect it approaches the dihydropyridine bases.

The HYDROCHLORIDE, $C_8H_{11}N.HCl$, forms white or slightly yellowish radiate masses which are deliquescent and very soluble in water. The hydrobromide, $C_8H_{11}N.HBr$, resembles it, but is less deliquescent and a trifle less soluble in cold water.

The PLATINOCHLORIDE, $(C_8H_{11}N.HCl)_2PtCl_4$, is a dark orange-colored powder, which is insoluble, or almost so, in cold water, and is a rather stable compound. Boiling water and water at 80° decompose it into hydrochloric acid and $(C_8H_{11}NCl)_2PtCl_2$, which is a light-brown powder, insoluble in cold, scarcely so in hot water.

The AUROCHLORIDE, $C_8H_{11}N.HCl.AuCl_3$, forms a light yellow precipitate. It is quite stable in cold, but very unstable in hot or even warm water. It cannot be modified by withdrawal of hydrochloric acid.

It forms two compounds with mercuric chloride. $(C_8H_{11}N.HCl)_2HgCl_2$ crystallizes in small white needles, which are slightly soluble in water and in dilute alcohol, insoluble in absolute alcohol, and on exposure to moist air undergo change. The second compound, $2(C_8H_{11}N.HCl).3HgCl_2$, is obtained by adding an excess of concentrated mercuric chloride to a concentrated solution of the hydrochloride. It forms slightly yellow, somewhat longer needles which are insoluble in the principal solvents, and are likewise changed by atmospheric humidity.

The IODOMETHYLATE, $C_8H_{11}N.CH_3I$, is formed by mixing solutions of the base and methyl iodide in absolute ether. It is deposited as a network of fine white needles, which are but slowly altered in the air, and are soluble in absolute alcohol. This solution on the addition of a little potash assumes a dark-red color, which is heightened by the addition of a little hydrochloric or acetic acid, and destroyed by ammonia without any resultant fluorescence. Warmed with excess of moist solid potash it becomes garnet-red in color and gives off an odor resembling that of the dihydropyridines. It thus behaves the same as the pyridine iodomethylates.

On oxidation with potassium permanganate it yields an acid which melts at 229°–230°, and begins to sublime at 150°. It presents all the characteristics of nicotinic acid, $C_6H_5NO_2$, which is formed as the result of oxidation of nicotine. With hydrochloric acid it forms the compound, $C_6H_5NO_2.HCl$. With copper acetate it forms a salt; this, distilled with lime, yields a substance which on boiling with platinum chloride and water forms the compound $(C_5H_5NCl)_2.PtCl_2$. This same substance forms an iodomethylate, which in alcoholic solution gives, on addition of potash, the characteristic reaction of pyridine bases.

The base, $C_8H_{11}N$, therefore yields pyridine and nicotinic acid.

A BASE, $C_8H_{13}N$, was obtained by GAUTIER and ETARD (1881) from the chloroformic extracts (see method, page 164) from putrefying mackerel, as well as from the decomposing flesh of the horse and ox. It is regarded by these authors as a constant and definite product of the bacterial fermentation of albuminoid substances, but this view is hardly justifiable, inasmuch as the base has not been found by other investigators. It is accompanied by the base $C_{17}H_{38}N_4$ (page 228). NENCKI (1882) asserted the identity of this base with the one which he had isolated in 1876, and to which he had ascribed the formula $C_8H_{11}N$. On the other hand GAUTIER and ETARD consider their base to be identical with the hydrocollidine obtained by CAHOURS and ETARD by the action of selenium on nicotine.

The free base is an alkaline, almost colorless, oily liquid, possessing a penetrating odor resembling that of syringa. It is volatile without decomposition, and boils at about 205°, while hydrocollidine boils at 210°. Its density at zero is 1.0296. When exposed to the air it oxidizes slowly, becomes brown and viscous, and at the same time absorbs carbonic acid. It differs from a collidine in possessing a strong reducing action, since both the gold and platinum double salts become reduced on heating, and even in the cold.

The HYDROCHLORIDE, $C_8H_{13}N.HCl$, is very soluble in water and in alcohol, and usually forms fine needles resembling snow crystals. It is neutral in reaction and possesses a bitter taste. In the presence of an excess of acid it reddens and resinifies.

The PLATINOCHLORIDE, $(C_8H_{13}N.HCl)_2PtCl_4$ ($Pt =$ 29.7 per cent.), is of a light yellow, flesh-color, crystalline, and but slightly soluble. It dissolves on warming, and recrystallizes in bent needles.

The AUROCHLORIDE is rather soluble, and becomes slowly reduced in the cold; rapidly on warming.

Physiological Action.—This isomer of hydrocollidine is strongly poisonous. Even so small a dose as 0.0017 gramme of the hydrochloride produced, when injected under the skin of a bird, marked unsteadiness of gait, followed by paralysis of the extremities, and finally death. The pupils are normal and the heart stops in diastole. Larger doses (0.007 gramme) cause at first vomiting and staggering, which soon give way to a condition of exaltation. Toward the end tetanic convulsions set in, followed by almost complete paralysis.

A BASE, $C_9H_{13}N$, isomeric with parvoline, has been extracted by GAUTIER and ETARD (1881) from decomposing mackerel and horseflesh. The method employed by these chemists for its isolation is given on page 164. The identity of this base with the synthetic parvoline, obtained by WAAGE by heating ammonia with propionic aldehyde in a sealed tube at 200°, cannot be considered to be definitely settled, although an apparent identity exists in regard to their boiling-points. Thus, the synthetic parvoline boils at 193°–196°, while GAUTIER and ETARD assign to their base a boiling-point a little below 200°. Further investigation is necessary to decide upon the question of the identity of this base with parvoline, or of the ptomaïne $C_8H_{13}N$ with hydrocollidine.

The free base is an oily, amber-colored liquid, possessing the odor of hawthorn blossoms. It is slightly soluble in water; very soluble in alcohol, in ether, and in chloroform. Its boiling-point, as stated above, is a trifle below 200°. Like the bases $C_8H_{13}N$ and $C_{10}H_{15}N$ it becomes brown and soon resinifies on exposure to air.

The PLATINOCHLORIDE, $(C_9H_{13}N.HCl)_2PtCl_4$ ($Pt =$ 28.65 per cent.), is slightly soluble, crystalline, and flesh colored; exposed to the air it soon becomes pink.

The AUROCHLORIDE is quite soluble.

A BASE, $C_{10}H_{15}N$, was isolated by GUARESCHI and MOSSO (1883) from ox-blood fibrin which had been allowed to putrefy for five months. In 1887 it was re-obtained from putrid fibrin by GUARESCHI, who this time ascribed to it the formula $C_{10}H_{13}N$. In 1886 OECHSNER DE CONINCK found it among the basic products formed in the putrefaction of the jelly-fish (*poulpes marins*, HUGOUNENQ, page 21). The method used for its extraction was that of GAUTIER and ETARD (see page 164). It forms a brownish oil of strong alkaline reaction, which soon resinifies. It possesses an unpleasant, weak pyridine or coniine odor, and is but slightly soluble in water; soluble in ether and in chloroform.

In regard to the constitution of this ptomaïne we know nothing, but from its physical characters it would seem to possess a pyridine nucleus. It is isomeric with corindine, a homologue of parvoline and collidine, which has been obtained from coal-tar.

For the behavior of the hydrochloride to alkaloidal reagents, see Table I.

The HYDROCHLORIDE, $C_{10}H_{15}N.HCl$, crystallizes in colorless cholesterine-like plates which are somewhat deliquescent.

The PLATINOCHLORIDE, $(C_{10}H_{15}N.HCl)_2PtCl_4$ ($Pt =$ 27.52 per cent.), forms a light flesh-colored, crystalline precipitate, and is insoluble in water, alcohol, and ether. It does not resinify, and is stable at 100°.

Physiological Action.—This ptomaïne resembles curara, although it is by no means as strong. 0.012 gramme of the free base produced in a frog dilatation of the pupil and slowing of the respiration. The nostrils were motionless, and within five hours complete paralysis of the muscles took place. The reflex excitability gradually diminished until it finally disappeared. An orange-blossom odor was observed about the frogs which were poisoned by this ptomaïne. The same amount of ptomaïne injected into a greenfinch produced vomiting, and a condition of weakness and decreased sensibility, followed soon, however, by recovery. A rat was not affected by 0.020 gramme of the free base. The hydrochloride acts much more energetically.

A BASE, $C_{10}H_{15}N$, was isolated by O. DE CONINCK, in 1886 (HUGOUNENQ, page 21, *C. Rendus*, 1888), from sea-polyps in an advanced stage of putrefaction, together with the base $C_8H_{11}N$. The method employed for its extraction was that of GAUTIER and ETARD (see page 164). It forms a slightly yellow, viscous liquid, and possesses a pleasant odor resembling that of blooming broom. Its density is about 1.18. It boils at about 230° (uncorrected), with initial decomposition. In water it is but slightly soluble, readily so in ether, alcohol, acetone, and ligroïn. It is rapidly oxidized by the air, becomes brown, and resinifies but does not absorb carbonic acid.

The HYDROCHLORIDE, $C_{10}H_{15}N.HCl$, forms fine yellowish, very deliquescent needles, which in the presence of a trace of air are at once colored red; if more air is present the red changes to a brown, and in the open air a resin is formed the same as from the free base. It is very easily soluble.

The HYDROBROMIDE, $C_{10}H_{15}N.HBr$, crystallizes in a network of fine deliquescent needles, which become likewise red on exposure to air. It is very soluble in water; less so in strong alcohol, and almost insoluble in ether.

The PLATINOCHLORIDE, $(C_{10}H_{15}N.HCl)_2PtCl_4$, forms a dark-red powder, which is insoluble in cold water; very soluble in warm water. It can be kept in dry air; in moist air it loses hydrochloric acid and becomes partially oxidized. Boiling water decomposes it. $(C_{10}H_{15}N.Cl)_2PtCl_2$ forms clear-brown plates, which are stable in moist air, and melt at 206°. It is insoluble in cold water, soluble in boiling water, but decomposes. In recrystallizing, warm previously boiled water should be used.

The AUROCHLORIDE, $C_{10}H_{15}N.HCl.AuCl_3$, occurs as a light-yellow precipitate; insoluble in cold water, soluble in warm water. It is decomposed by boiling water; is stable when kept in a moist atmosphere.

The IODOMETHYLATE, $C_{10}H_{15}N.CH_3I$, in warm alcoholic solution yields, on the addition of strong potash, a bright-red color, which soon becomes brown, and in about an hour the solution shows a greenish-blue fluorescence. This rapidity of change is due to the extreme oxidizability of the ptomaïne.

O. DE CONINCK considers this base, as well as $C_8H_{11}N$, as belonging to the pyridine and not to the hydropyridine series.

A BASE, $C_{10}H_{17}N$, was described by GRIFFITHS (1890) as derived from cultures on peptone-agar of the bacterium allii, a germ obtained from putrid onions. The base (hydrochloride?) forms colorless, prismatic, microscopic, very deliquescent needles, which are soluble in warm water, alcohol, ether, and chloroform. It gives off a hawthorn-like odor, especially when warmed. With phosphomolybdic acid it yields a white; with iodine in potassium iodide and with tannic acid a chestnut-colored precipitate. NESSLER'S solution produces a yellow chestnut-colored precipitate. Picric acid throws down a yellow slightly soluble deposit. The platinochloride, $(C_{10}H_{17}N.HCl)_2PtCl_4$, is yellow, crystalline, and difficultly soluble in cold water and in alcohol; soluble in warm water. Gold chloride produces a thick yellow precipitate soluble in water. Dilute sulphuric acid produces a *violet-red* color. The base is apparently a hydrocoridine.

A BASE, $C_{32}H_{31}N$, was obtained by DELÉZINIER (1889) and is said to be the alkaloid, isolated in 1879 by BROUARDEL, which in its chemical and physiological properties was described as similar to veratrine. It forms an almost colorless oily fluid, which possesses a hawthorn-like odor. It is very readily oxidizable and yields the veratrine-like reactions only in the presence of air. It is soluble in alcohol, ether, toluene, and benzene; and forms well-defined salts which are very deliquescent. It appears to be an amine, and in its composition differs from cevadine by $9H_2O$. Nothing is stated in regard to its source or method of preparation. The analytical results given—$C = 89.41$, $H = 7.3$, $N = 3.03$—correspond more to the formula $C_{34}H_{33}N$.

ETHYLIDENEDIAMINE (?), $C_2H_8N_2$.—This base was considered at first by BRIEGER to be identical with ethylenediamine, but subsequent comparison showed this to be an error. Thus, the former is poisonous and does not form a gold salt, while the latter is not poisonous and does form a rather difficultly soluble gold salt. Again, ethylenediamine forms a platinochloride which is almost insoluble in hot water, whereas the platinum double salt of the ptomaïne is much more easily soluble. **Brieger** is, therefore, inclined to think that it is identical with ethylidenediamine, $CH_3.CH(NH_2)_2$, rather than with ethylenediamine, which has the structure, $CH_2.NH_2.CH_2.NH_2$. This ptomaïne was obtained by **Brieger**, in 1885 (I., 44), from decomposing haddock (see Gadinine).

The free base can be obtained, without decomposition, on distilling the hydrochloride with sodium hydrate.

The **Hydrochloride**, $C_2H_8N_2.2HCl$, crystallizes in long glistening needles which are readily soluble in water, insoluble in absolute alcohol. It gives no combination with gold chloride. For its behavior to alkaloidal reagents see Table I.

The **Platinochloride**, $C_2H_8N_2.2HCl.PtCl_4$ (Pt = 41.49 per cent.), forms small yellow plates which are moderately difficultly soluble in water. It can be readily recrystallized from hot water.

Physiological Action.—Frogs seem to be less susceptible to the action of this poison than mice or guinea-pigs. In the latter, it produces a short time after injection an abundant periodic flow of secretion from the nose, mouth, and eyes. The pupils dilate and the eyeballs project. Violent dyspnœa then comes on and predominates until the death of the animal, which does not take place for twenty-four hours or more. The heart is stopped in diastole.

Trimethylenediamine (?), $C_3H_{10}N_2$ (?), is a toxic base isolated by **Brieger** (1887) from cultures of the comma bacillus on beef-broth. It may be stated here that from the same source, cholera cultures, **Kunz** (1888) obtained a base which he considered to be identical with spermine or ethyleneimine (see next chapter). It is present, however, in exceedingly minute quantity, and occurs in the mercuric chloride precipitate, from which it is obtained by the following method: The precipitate is decomposed by hydrogen sulphide, the filtrate evaporated to dryness, and the residue taken up with absolute alcohol and precipitated by an alcoholic solution of sodium picrate. The precipitate thus obtained consists of the picrates of cadaverine, creatinine, and of this new base. It is boiled with absolute alcohol to remove the insoluble cadaverine picrate; the filtrate is evaporated to expel the alcohol, and the bases then converted into the platinum double salts, whereby the easily soluble creatinine platinochloride can be separated from the corresponding less soluble compound of the new base.

Owing to the small quantity of this substance present, a complete study of its properties has not as yet been made. It gives difficultly soluble precipitates with gold chloride and with platinum chloride; the compound with the latter crystallizes in long needles. With picric acid it gives a precipitate consisting of felted needles, which resemble creatinine picrate; they melt at 198°. Phosphomolybdic acid yields a precipitate crystallizing in plates, while potassium-bismuth iodide gives dark-colored fine needles. From its physiological action it seems to be identical with the basic substance isolated from choleraic bodies by different observers. It causes violent convulsions and muscle tremor.

Besides trimethylenediamine another toxine was obtained by BRIEGER from cholera cultures, but in quantity insufficient for analysis. It was obtained from the mercuric chloride filtrate after elimination of methylamine, trimethylamine, and traces of choline and creatinine, as an insoluble platinum double salt. Subcutaneous injection of this base into mice produced a paralysis-like lethargic condition, slowing of respiration and heart's action, lowering of temperature, and finally, death in twelve to twenty-four hours. In some cases bloody stools were passed.

PUTRESCINE, $C_4H_{12}N_2$, is a diamine which almost invariably occurs together with cadaverine, with which it is apparently closely related. This base was also discovered by BRIEGER in 1885 (II., 42), who has obtained it from putrefying human internal organs (for four months at a low temperature without access of much oxygen); and from the same material, decomposing at the ordinary temperature of the room, for from three days to three weeks. It has also been obtained from herring, twelve days in spring; from pike, six days in summer; from haddock, two months (**Bocklisch**). Also from putrid mussel, sixteen days (**Brieger**); and from human as well as horse flesh. **Brieger** has obtained it from cultures of the bacteria of human feces on gelatin, and in small quantity in rather old cultures of the comma bacillus on beef-broth; in larger quantity in cultures of the same germ on blood-serum.

Udránszky and **Baumann** in 1888 demonstrated the existence of putrescine and cadaverine in the urine of cystinuria, the former constituting about one-third of the total amount of the two bases present. In the feces of the same patient, on the contrary, putrescine constituted by far the greater quantity, while cadaverine formed but 10 to 15 per cent. Normal feces, as well as the feces of various diseases with the possible exception of cholera stools, are free from diamines. It would seem, therefore, that these bases occur in cystinuria as the result of putrefactive changes going on in the intestines; becoming partly absorbed they appear in the urine. In two cases of cystinuria, reported by **Brieger** and **Stadthagen**, cadaverine was found almost solely present in the urine.

According to **Mester** the diamines are proportionate to the amount of cystin excreted, and therefore constitute a fixed symptom, the cause of which is the same as that of the cystinuria.

Although putrescine is recognizable on about the fourth day of the putrefaction, yet it does not occur in appreciable quantity until about the eleventh day. The amount that is formed increases as the putrefaction goes on, so that a considerable quantity may be obtained after two or three weeks. A very good source for the preparation of putrescine, cadaverine, and neuridine is gelatin which has been allowed to decompose in contact with water for some weeks. Neuridine is, apparently, formed first, but is soon replaced by the former two bases. In the process of extraction it is first obtained in the alcoholic mercuric chloride precipitate. For its separation from cadaverine and other accompanying bases, see page 220.

From the urine of cystinuria it is best obtained by precipitation with benzoyl chloride (BAUMANN's method). For this purpose about 1500 c.c. of urine are treated with 200 c.c. of sodium hydrate solution (10 per cent.), then 20 to 25 c.c. of benzoyl chloride is added, and the whole shaken till the odor of the latter disappears. The yellowish-white precipitate which forms may consist of insoluble phosphates, carbohydrates, polyatomic alcohols, and diamines. The cystin compound is precipitated only in concentrated solutions. The precipitate contains from a half to two-thirds of the diamines present; it is filtered off, digested with warm alcohol, and the solution filtered. The alcoholic filtrate is concentrated and then poured into about thirty times its volume of cold water. The diamine compounds then crystallize out. To separate the two diamines they are redissolved in just sufficient warm alcohol to effect solution, and this is then poured into about twenty times this volume of ether. The putrescine benzoyl compound is thus thrown out of solution. The filtrate from this on concentration yields the cadaverine compound. To isolate that portion of the diamines which remained in the original filtrate with benzoyl cystin, it is acidulated with sulphuric acid and extracted with ether. The residue obtained on evaporating the ethereal solution is first neutralized with a 12 per cent. sodium hydrate solution, then mixed with three to four times its volume of the same solution. The precipitate which forms consists of the sodium compounds of benzoyl cystin and the diamines. It is washed with sodium hydrate, and the two compounds separated by their different solubilities in water—the cystin compound is readily soluble, that of the diamines insoluble. To purify the benzoyl diamines they are dissolved in warm alcohol and precipitated with excess of water.

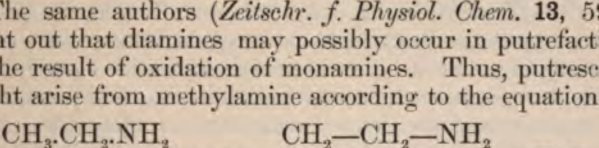
Putrescine (from *putresco*, to rot, to putrefy) is a water-clear, rather thin liquid which fumes in the air and has a a peculiar semen-like odor, almost undistinguishable from that of cadaverine, and reminding one somewhat of the pyridine bases. It absorbs carbonic acid energetically from the air, without losing thereby the repulsive odor. The boiling-point of the free base, as ordinarily obtained, is about 135°. It is not decomposed by distillation with potassium hydrate, and is rather difficultly volatile with steam. With acids it forms beautiful crystalline salts. Putrescine unites with water, like ethylenediamine, to form a hydrate, and this water can only be removed by distillation with metallic sodium. The perfectly anhydrous base boils at 156°–157°, and then solidifies to plates (BRIEGER), which melt at 24° (UDRÁNSZKY and BAUMANN). The synthetic base boils at 158°–160°, and melts at 23°–24° (LADENBURG). Like cadaverine it is difficultly soluble in ether.

The constitution of putrescine has been determined by UDRÁNSZKY and BAUMANN (1888). They showed that the dibenzoyl compound of putrescine was identical with that of the synthetic tetramethylenediamine and of the base which they found in the urine of cystinuria.

Putrescine, therefore, is tetramethylenediamine, a homologue of cadaverine, and its rational formula is :

$$\mathrm{NH_2.CH_2.CH_2.CH_2\ CH_2.NH_2.}$$

The same authors (*Zeitschr. f. Physiol. Chem.* **13**, 591) point out that diamines may possibly occur in putrefaction as the result of oxidation of monamines. Thus, putrescine might arise from methylamine according to the equation :



$$\begin{array}{l}\mathrm{CH_3.CH_2.NH_2}\\ \mathrm{CH_3.CH_2.NH_2}\end{array} + \mathrm{O} = \begin{array}{l}\mathrm{CH_2{-}CH_2{-}NH_2}\\ |\\ \mathrm{CH_2{-}CH_2{-}NH_2}\end{array} + \mathrm{H_2O.}$$

In a similar manner cadaverine might form from ethyl and propylamine.

Putrescine can be prepared synthetically, according to LADENBURG's method, by converting ethylene bromide into the cyanide and then reducing this by means of sodium in absolute alcohol.

On heating the concentrated aqueous solution of the hydrochloride with potassium nitrite there is produced an oil, soluble in water, from which it can be extracted with ether. This oil, on treatment with phenol and sulphuric acid, gives LIEBERMANN's nitroso-reaction, which would seem to show that putrescine is not a primary diamine (butylenediamine), but is rather a secondary diamine (BRIEGER, II., 42). As a primary diamine it should take up, on repeated treatment with methyl iodide, six methyl radicals; whereas, if it is a secondary diamine, only four methyl radicals can enter the molecule. Thus, to illustrate, methylamine, $CH_3.NH_2$ (a primary amine), combines with three molecules of methyl iodide to form $(CH_3)_4N.HI$. Similarly, dimethylamine $(CH_3)_2.NH$, requires only two molecules to form $(CH_3)_4N.HI$. In the case of diamines, double this number of methyl groups is required to effect complete saturation. As a matter of fact, BRIEGER (III, 101), on treating putrescine with methyl iodide, has succeeded in introducing four, and only four, methyl radicals. From this, however, it does not follow that putrescine is not a primary amine, since cadaverine, an unquestioned primary diamine, yields a substitution compound containing only two methyl groups (see p. 215).

The tetra-methyl substitution-product of putrescine, $C_4H_8(CH_3)_4N_2$, can be distilled without decomposition. The free base crystallizes in long prisms. The hydrochloride forms small needles which are easily soluble; with phosphotungstic acid it gives a white crystalline precipitate, with phosphomolybdic acid a yellow crystalline precipitate, with picric acid needles. Potassium-bismuth iodide gives a brownish-red amorphous deposit, while the potassium mercuric iodide forms prisms. Gold chloride yields difficultly, and platinum chloride easily soluble octahedra; aqueous mercuric chloride forms needles.

The aurochloride has the formula $C_8H_{22}N_2.2AuCl_4$.

This tetra-methyl derivative of putrescine is enormously poisonous as compared with putrescine. The symptoms are the same as those produced by muscarine or neurine. They are: abundant salivation; dyspnœa—respiration at first increases, then decreases; contraction of the pupils; paralysis of the muscles of the limbs and trunk; increased peristaltic action of the intestines, ejaculation of semen, dribbling of urine, and, finally, violent clonic convulsions. In the case of mice and guinea-pigs the convulsions are prominent immediately after the injection of the poison.

PUTRESCINE HYDROCHLORIDE, $C_4H_{12}N_2.2HCl$, forms long colorless needles, which are very easily soluble in water; difficultly so in dilute alcohol; entirely insoluble in absolute alcohol, and can thus be separated from cadaverine hydrochloride. To accomplish this separation it is, perhaps, better to dissolve the mixture of the hydrochlorides in hot 96 per cent. alcohol. On cooling the solution thus obtained, the putrescine salt crystallizes out, whereas that of cadaverine remains in solution. Putrescine hydrochloride differs from cadaverine hydrochloride in that it is not hygroscopic and can be exposed for days to the air without suffering any change on the surface of the crystals.

For the behavior of the free base and the hydrochloride to alkaloidal reagents, see Table I. Putrescine is not toxic, though it possesses some marked physiological properties (see Cadaverine, page 215). According to SCHEURLEN putrescine, like cadaverine, produces inflammation, suppuration, and necrosis. It is not poisonous to dogs (UDRÁNSZKY and BAUMANN). It is optically inactive.

The PLATINOCHLORIDE, $C_4H_{12}N_2.2HCl.PtCl_4$ ($Pt = 39.16$ per cent.), often appears under the microscope in the form of cholesterine-like plates. In the pure condition it appears as six-sided plates, which are superposed in layers. The crystals possess a splendid silvery lustre, and are rather difficultly soluble in cold water; less so in hot water.

The AUROCHLORIDE, $C_4H_{12}N_2.2HCl.2AuCl_3 + 2H_2O$, crystallizes likewise in plates, which are difficultly soluble in cold water. It can, therefore, be readily separated from cadaverine aurochloride, which is easily soluble in water. The water of crystallization can be driven off completely only at 110° (BRIEGER). According to BOCKLISCH, it loses this water on standing over sulphuric acid, or on heating at 100°.

The PICRATE, $C_4H_{12}N_2.2C_6H_2(NO_2)_3OH$, is difficultly soluble, and crystallizes from a hot aqueous solution in needles; from hot aqueous alcohol, on cooling, in yellow plates. It begins to brown at 230°, and on further heating becomes darker, till finally, at 250°, it decomposes with rapid evolution of gas (BOCKLISCH).

The CARBONATE is crystalline.

The MERCURY DOUBLE SALT is easily soluble in a large quantity of water, and can thus be separated from the cadaverine salt, which is difficultly soluble. From hot concentrated aqueous solution it crystallizes in needles.

The DIBENZOYL-PUTRESCINE, $C_4H_8(NHCOC_6H_5)_2$, forms silky plates or long needles, which are more difficultly soluble in hot alcohol than those of the cadaverine compound. From this solution it is reprecipitated by addition of water or ether. Its melting-point is 175°. It sublimes without decomposition.

CADAVERINE, $C_5H_{14}N_2$, is a diamine isomeric with saprine and neuridine, and, like the latter, it occurs very frequently in decomposing animal tissues. Twelve isomers of this composition are possible. Another isomer, gerontine (see next chapter) has been described by GRANDIS (1890). It is a very striking fact, that in ordinary putrefaction as choline disappears the diamines appear and increase in quantity according as the time of putrefaction is extended. It is also worthy of note that cadaverine appears in putrefaction before putrescine. It has been obtained by BRIEGER (1885) from human lungs, hearts, livers, etc. (hence the name), which were allowed to putrefy at the ordinary temperature for three days; from the same organs, and from horseflesh, after four months in a closed vessel at —9° to + 5°; from putrid mussel after sixteen days; from putrid egg and blood albumin. It seems to be a constant product of the growth of the comma bacillus, irrespective of the soil on which it is cultivated.

BOCKLISCH has isolated it from perch and pike, six days in midsummer; from herring, twelve days in spring; from haddock, two months at a low temperature; from cultivations of FINKLER and PRIOR'S vibrio proteus on beef-broth, thirty to thirty-five days at 37° to 38° (*Ber.* **20**, 1441). Cadaverine seems to be a constant product of the activity of the genus vibrio, inasmuch as it does not occur in cultures in which this genus is absent. Thus, it is not present in the excrements of healthy or typhoid patients; in cultures of EMMERICH's bacillus, of EBERTH's bacillus, and of the pyogenic bacteria. It is said to occur in cultures of the bacillus of hog-cholera (V. SCHWEINITZ). OECHSNER DE CONINCK has found it in putrid jelly-fish (HUGOUNENQ, page 23). It is present with putrescine in the urine and feces of cystinuria (UDRÁNSZKY and BAUMANN, 1888). The odor of cholera stools and the breath of cholera patients may be possibly due to cadaverine, although the base has not been demonstrated in such cases. It has also been obtained from caviar.

Cadaverine occurs in the mercuric chloride precipitate, from which it is isolated according to the methods given on pages 206 and 221. For its isolation and separation from putrescine by the use of benzoyl chloride, see page 208.

This base was at first ascribed the formula $C_5H_{16}N_2$, but subsequent researches led BRIEGER and BOCKLISCH to the adoption of the formula $C_5H_{14}N_2$. In 1883, LADENBURG prepared, as the first step in the synthesis of piperidine, a base, pentamethylenediamine, possessing the same empirical formula as cadaverine, and later (*Ber.* **18**, 2956) he showed the possibility of the identity of these two bases. This led to their direct comparison and the successful establishment of their identity. In fact, LADENBURG, as a crucial test of the identity, converted cadaverine into piperidine, and found the latter base to agree entirely in its chemical and physical properties with those of the natural alkaloid (*Ber.* **19**, 2586). LADENBURG, however, observed one apparent difference between cadaverine and pentamethylenediamine, and that was in the composition of the mercury double salts. That of the former base, whether obtained from alcoholic or aqueous solution (BOCKLISCH, *Ber.* **20**, 1441), was found to combine with four molecules of mercuric chloride; whereas the double salt of pentamethylenediamine was found by LADENBURG to contain only three molecules of mercuric chloride. Subsequently he found that he had prepared this salt by mixing the aqueous solutions of the hydrochloride of the base and of the mercuric chloride in the molecular ratio of 1 to 4, and on using a larger excess of mercuric chloride he obtained a salt containing four molecules of mercuric chloride (*Ber.* **20**, 2216). The complete identity of these two bases has, therefore, been established. The constitutional formula of cadaverine is, therefore:

$$NH_2—CH_2—CH_2—CH_2—CH_2—CH_2—NH_2.$$

Cadaverine can be prepared synthetically according to LADENBURG'S method. For this purpose trimethylene bromide is converted into the cyanide, and this is then reduced by sodium in absolute alcohol.

Cadaverine forms a somewhat thick, water-clear, syrupy liquid, which possesses an exceedingly unpleasant odor, resembling somewhat that of coniine (piperidine) and of semen. When dehydrated with potassium hydrate it boils at 115°–120° (BRIEGER). It boils at 175° (BRIEGER, III., 98), and fumes in the air. The base eagerly absorbs carbonic acid from the air, and solidifies into a crystalline mass, the carbonate. It is volatile with steam, and can be distilled, without decomposition, even in presence of sodium or barium hydrate, or soda lime. Neuridine, its isomer, decomposes under these circumstances. When heated with alcoholic potash and chloroform it does not give the isonitril reaction, nor does it give the characteristic odor of oil of mustard on treatment with carbon disulphide and mercuric chloride. The absence of these reactions at first induced BRIEGER to conclude that cadaverine and putrescine were not primary amines, but LADENBURG (1885) showed that this conclusion was not justifiable. These two reactions are given by primary monamines, but in this case they are not given by cadaverine, a primary diamine. It is probable that this behavior holds true for all diamines.

Cadaverine is, undoubtedly, identical with the so-called "animal coniine," which has been isolated at various times from cadavers.

Cadaverine and putrescine were at first regarded as physiologically indifferent, but more recent investigations by SCHEURLEN, GRAWITZ, and others, show that both these bases are capable of producing strong inflammation and necrosis. According to BEHRING, in large doses it is poisonous to mice, rabbits, and guinea-pigs; it is not poisonous to dogs (UDRÁNSZKY and BAUMANN). Cadaverine is one of those substances which can set up suppuration in the absence of bacteria. In cholera Asiatica the necrosis of the intestinal epithelium is quite common, and it would seem that this pathological change, as well as the muscular spasms and algidity, are due to the presence of these bases. It should be noted, however, that UDRÁNSZKY and BAUMANN failed to obtain any sign of intestinal irritation on feeding dogs enormous doses of cadaverine. Besides these local effects, they prevent, even in small quantity, the coagulation of blood, and render it "laky." According to GRAWITZ, cadaverine seems to hinder the growth of bacteria. The cystitis observed in cystinuria may possibly be due to the presence of cadaverine and putrescine in the urine. Both bases are optically inactive.

When cadaverine is treated with methyl iodide, a base is obtained, the hydrochloride of which gives with platinum chloride a double salt, having the composition: $C_5H_{12}(CH_3)_2N_2.2HCl.PtCl_4$. This new base, therefore, is cadaverine in which two atoms of hydrogen have been replaced by two methyl radicals. The platinochloride of this derivative forms long, clear red needles, which, unlike those of cadaverine, do not change their shape on repeated recrystallization. It is moderately difficultly soluble in water (BRIEGER, II., 41). Since cadaverine is a primary diamine it should combine with six molecules of methyl iodide to form a saturated compound. This, however, has not been obtained.

The HYDROCHLORIDE, $C_5H_{14}N_2.2HCl$, crystallizes in beautiful, long deliquescent needles (BRIEGER). According to BOCKLISCH, it forms long, colorless needles or prisms; crystallizes from alcohol in plates, and is not deliquescent except on long standing. It is soluble in water, alcohol,

alcohol-ether; but is insoluble in absolute alcohol, ether, etc. It can readily be separated from putrescine hydrochloride by its solubility in 96 per cent. alcohol (Bocklisch). The strictly pure base, as well as the hydrochloride, does not give a blue color with ferric chloride and potassium ferricyanide. For reactions of the hydrochloride and of the free base, see Table I.

Cadaverine hydrochloride on dry distillation decomposes into NH_3, HCl, and piperidine, $C_5H_{11}N$. The latter is a well-known poisonous alkaloid which exists in the combined state in black pepper. It is not known whether this change, whereby the non-poisonous cadaverine is converted into a toxic base, can take place under the influence of bacteria during the processes of putrefaction or not. However, it does not seem improbable that this simple chemical change should be effected through the action of living organisms; for Schmidt has already shown that the almost physiologically indifferent choline, when subjected to the action of the bacteria of hay-infusion, decomposes into a neurine-like base possessing a muscarine-like action, and under certain conditions it yields a base which in its action resembles pilocarpine.

The Sulphate likewise forms beautiful, well-formed needles, and in its solubility corresponds to the hydrochloride.

The Platinochloride, $C_5H_{14}N_2.2HCl.PtCl_4$ (Pt = 38.08 per cent.), crystallizes after some time, on the addition of platinum chloride to a not too concentrated solution of the hydrochloride, in the form of long, beautiful orange-red needles (Bocklisch). Ordinarily it is obtained at first in long, dirty red needles, which on repeated recrystallization become clearer and assume a form similar to that of ammonium platinochloride. It forms chrome-yellow rhombic prisms which are short and octahedra-like. In polarized light they are strongly double refracting. It is very slightly soluble in cold water; can be recrystallized from hot water (Bocklisch). Its solubility in water at 12° is 1 to 113–114. It decomposes at 235°–236°.

The Aurochloride, $C_5H_{14}N_2.2HCl.2AuCl_3$ (Au=50.41

per cent.), crystallizes partly in cubes, and partly in long needles which at first possess a bright lustre, but under the desiccator soon effloresce and become opaque. The water of crystallization is completely removed on standing over sulphuric acid. It is very easily soluble, and melts at 188° (BOCKLISCH).

The PICRATE, $C_5H_{14}N_2.2C_6H_2(NO_2)_3OH$, forms yellow plates which are difficultly soluble in cold water. From hot water it crystallizes in long prisms, which melt at 221° with decomposition. It is insoluble in absolute alcohol and can be recrystallized from hot dilute alcohol.

Cadaverine hydrochloride combines with mercuric chloride, when the aqueous solutions of these two salts are mixed in the molecular ratio of 1 to 4, to form $C_5H_{14}N_2.2HCl.3HgCl_2$. This salt can be recrystallized from hot water (LADENBURG). When an excess of mercuric chloride is used the double salt has the composition $C_5H_{14}N_2.2HCl.4HgCl_2$. This last salt melts at 216° (LADENBURG); at 214° (BOCKLISCH). It is difficultly soluble in cold water; from hot water it crystallizes in needles or plates (BOCKLISCH).

The NEUTRAL OXALATE, $C_5H_{14}N_2.H_2C_2O_4+2H_2O$, was prepared by BOCKLISCH by adding a little less than the calculated quantity of alcoholic oxalic acid to the cadaverine. The precipitate may be recrystallized from hot dilute alcohol, when it is obtained in the form of needles, which melt at about 160° and at the same time give off gas.

The ACID OXALATE, $C_5H_{14}N_2.2H_2C_2O_4+H_2O$, is made by bringing the neutral salt into alcoholic oxalic acid. It is soluble in hot dilute alcohol, and recrystallizes from it in quadratic plates, sometimes in glistening needles. It melts at 143° with decomposition. After it has been dried over sulphuric acid, it loses, on being heated to 105°–110°, one molecule of water (BOCKLISCH, *Ber.* **20**, 1441). The insolubility of these oxalates in absolute alcohol shows the fallacy of TAMBA's distinction between ptomaïnes and vegetable alkaloids. (See page 186.)

The DIBENZOYL derivative, $C_5H_{10}(NHCOC_6H_5)_2$, crystallizes in long needles and plates, readily soluble in alcohol, difficultly so in ether, and insoluble in water; hence the alcoholic solution can be precipitated by addition of water or ether (separation from the putrescine compound, see p. 208). It melts at 129°–130°. It is not changed by boiling with dilute acids and alkalies; but boiling with concentrated hydrochloric or sulphuric acids for a long time finally breaks it up.

NEURIDINE, $C_5H_{14}N_2$, was the first diamine isolated from animal tissues (BRIEGER, 1883). It is one of the most common products of putrefaction, and as such has been obtained by BRIEGER from putrid horseflesh, beef, human muscle, five to six days; from haddock, five days in summer; from cheese, six weeks in summer; from gelatin, ten days at 35°; from decomposing human internal organs, three to eleven days; from cultures of the EBERTH bacillus, with mydine.

BOCKLISCH has obtained it from perch, six days in summer; from barbel after three days in summer.

It has also been obtained from fresh eggs in the preparation of choline by heating with baryta; and also from fresh brain by heating with 2 per cent. hydrochloric acid (BRIEGER, I., 57–61). EHRENBERG (1887) found it in poisonous sausage and obtained it by growing a bacillus from this source on liver and meat bouillon.

Neuridine is almost invariably accompanied by choline, and as the duration of putrefaction increases, the latter gradually decreases in amount and yields a corresponding increase in trimethylamine, whereas the yield of neuridine increases from day to day. The amount of neuridine formed depends upon the nature of the organ employed in putrefaction. The greatest yield is obtained from gelatinous tissues, such as intestines; and especially from pure gelatin. On the other hand, such tissues as the spleen and liver yield but little.

Neuridine comes down in the mercuric chloride precipitate (sometimes it occurs in the filtrate), and can then be isolated from the other bases present in a number of ways. One method is given under Gadinine. Another convenient method of separation is to precipitate it from alcoholic solution by alcoholic picric acid. The picrate thus obtained is, for the purpose of further purification, recrystallized from absolute alcohol, then decomposed by extracting its acid solution with ether (to remove the picric acid) and evaporating the aqueous solution to dryness. The residue is now extracted with alcohol and the alcoholic solution precipitated by alcoholic platinum chloride. The platinochloride can now be recrystallized from hot water.

The free base, as obtained by the treatment of the hydrochloride with moist freshly precipitated silver oxide, possesses an extremely repulsive odor, similar to that of human semen. On evaporation of its aqueous solution it yields a gelatinous-like mass, and at the same time slowly decomposes. It does not crystallize when evaporated in a vacuum, and decomposes even under these conditions. The same disagreeable odor is obtained when the hydrochloride is warmed with potassium hydrate. BRIEGER (I., 24) regards this decomposition-product of neuridine as an oxidation product of the original substance.

The free base is very readily soluble in water, but is insoluble in ether and absolute alcohol; difficultly soluble in amyl alcohol. It gives white precipitates with mercuric chloride, neutral and basic lead acetates. When distilled with fixed alkali it yields di- and tri-methylamine, thus probably showing some relation to neurine, hence the name neuridine. It does not give HOFMANN's iso-nitril reaction, but it does not follow from this, as shown under cadaverine, that it may not be a primary diamine. It is isomeric with cadaverine, saprine and gerontine.

The HYDROCHLORIDE, $C_5H_{14}N_2.2HCl$, crystallizes in long needles which are extremely soluble in water and in dilute alcohol, but are insoluble in absolute alcohol, ether, benzol, chloroform, petroleum ether, benzine, amyl alcohol, etc. Its insolubility in absolute alcohol may be used to effect a separation from choline hydrochloride. It can be recrystallized from slightly warm dilute alcohol. Although the pure salt is insoluble in the reagents just given, nevertheless, in the presence of other animal matter it is dissolved in greater or less quantity, and hence can be obtained by the STAS-OTTO as well as by the DRAGENDORFF method. The crystals resemble urea in form. On heating very cautiously the salt sublimes, and at the same time appears to undergo a partial internal decomposition, inasmuch as many of the groups of needles in the sublimate are colored red or blue. For the behavior of the hydrochloride with the alkaloidal reagents, see Table I.

Pure neuridine is not poisonous, but as long as it is contaminated with other putrefaction products it possesses a toxic action similar to that of peptotoxine. This holds true for the other non-poisonous bases.

The PLATINOCHLORIDE, $C_5H_{14}N_2.2HCl.PtCl_4$, crystallizes in beautiful flat needles. Recrystallized from hot water, it forms aggregations of small, clear, yellow needles. It is readily soluble in water, from which it is precipitated on the addition of alcohol.

The AUROCHLORIDE, $C_5H_{14}N_2.2HCl.2AuCl_3$, is rather difficultly soluble in cold water (BOCKLISCH), and crystallizes on cooling of the hot, saturated solution in bunches of clear, yellow, short needles.

The PICRATE, $C_5H_{14}N_2.2C_6H_2(NO_2)_3OH$, can be recrystallized from boiling water, in which it is very difficultly soluble, in the form of needles united in plumose groups. It is almost insoluble in cold water; less difficultly soluble in alcohol. It is not fusible, but begins to brown and give off yellow vapors at 230°, and carbonizes completely at 250°.

SAPRINE, $C_5H_{14}N_2$, was found in human livers and spleens after three weeks' putrefaction (BRIEGER, II., 30, 46, 58). It occurs together with cadaverine, putrescine, and mydaleine in the mercuric chloride precipitate. To separate these bases, BRIEGER (1885) used the following process: The mercury salts were decomposed with hydrogen sulphide, the filtrate evaporated to dryness, and the residue then extracted with alcohol. The putrescine hydrochloride is insoluble in alcohol, and is thus removed. The alcoholic solution was treated with platinum chloride, which precipitated the greater part of the cadaverine. The mother-liquor, on concentration, yielded a mixture of the platinochlorides of cadaverine and saprine. Each successive crop contained more of the saprine double salt. The two kinds of crystals were now separated by means of a magnifying-glass. The saprine platinochloride thus obtained was finally purified by repeated recrystallization from water. The mother-liquor, after the removal of the saprine platinochloride, contains the mydaleine salt, which, on account of its solubility in water, crystallizes only on concentration, or on standing under a desiccator. The mercuric chloride filtrate contains some mydaleine and the ptomaïne, which yields a platinochloride containing 28.40 per cent. platinum.

The free base is a diamine, and was first ascribed the formula $C_5H_{16}N_2$. It appears, however, to be isomeric with cadaverine and neuridine. The term saprine is derived from the Greek σαπρός, signifying putrid. It possesses a weak pyridine-like odor, and can be distilled with steam or with potassium hydrate without undergoing decomposition. In its reactions it behaves the same as cadaverine, except that it gives an amorphous precipitate with potassium-bismuth iodide, whereas cadaverine gives a crystalline precipitate. The free base gives an immediate intense blue color with ferric chloride and potassium ferricyanide.

The HYDROCHLORIDE, $C_5H_{14}N_2.2HCl$, forms flat needles which are not hygroscopic (distinction from cadaverine hydrochloride). Its reactions are the same as those of cadaverine hydrochloride (see Table I.). It is, however, tinged slightly blue by a mixture of ferric chloride and potassium ferricyanide, whereas the free base gives an intense blue. It differs from cadaverine in that it does not give the reddish-brown color with potassium bichromate and sulphuric acid. Again, it forms no aurochloride; while, on the other hand, cadaverine hydrochloride yields an easily soluble salt, crystallizing in splendid needles.

The PLATINOCHLORIDE, $C_5H_{14}N_2.2HCl.PtCl_4$, forms parallel, aggregated, pointed crystals, which are somewhat soluble in water, and are thus distinguished from cadaverine platinochloride, which crystallizes in rhombs, and is difficultly soluble in water.

Physiologically, it is indifferent.

A Base, $C_7H_{10}N_2$.—Until very recently the nature of the basic substances which are formed as products of the alcoholic fermentation of sugar or molasses has been but little understood. Krämer and Pinner, in 1869, found in crude fusel oil a small quantity of a volatile base which they apparently identified with a collidine. This observation was confirmed by Ordonneau and others; and still more recently (January, 1888) Morin has contributed an elaborate paper upon the bases formed during alcoholic fermentation. The portion of crude fusel oil which boils above 130.5° was extracted with slightly acidulated water, the acid aqueous solution thus obtained was made alkaline, and the oily bases which were thus set free were then distilled with vapor of water. The free bases were dried over potassium hydrate and then subjected to fractional distillation. Three fractions were thus obtained, boiling respectively at 155°–160°, 171°–172°, and 185°–190°. Only the second fraction, which boils at 171°–172°, was studied, and was found to possess the formula $C_7H_{10}N_2$. Heated with concentrated hydrochloric acid, it is decomposed in part with the formation of ammonia. It combines with ethyl iodide to form a yellow crystalline compound, which is soluble in water and alcohol, insoluble in ether. The hydrochloride crystallizes in fine white needles, soluble in water and alcohol, and but very slightly soluble in absolute ether. The free base, as stated above, boils at 171°–172°, is very soluble in water, alcohol, ether, etc. When pure it forms a colorless, strongly refracting, very mobile oil, which possesses a characteristic nauseating odor, but slightly resembling that of the pyridine bases. Its density at 12° is 0.9826; toward litmus paper the base shows no decided reaction. The platinochloride is crystalline and is very soluble in water and alcohol, slightly soluble in ether. Potassio-mercuric iodide does not precipitate the aqueous solution of the free base, but in solutions of the hydrochloride it gives a yellow flocculent precipitate, which soon crystallizes in long brilliant yellow needles. This reaction takes place readily in solutions of 1 to 1000, and only after some hours in solutions of 1 to 10,000; and is not given by the bases of the pyridic and quinolinic series. Mercuric chloride produces an immediate flocculent precipitate in solutions of the base having a concentration of 1 to 1000, but requires some time to appear in 1 to 10,000. Phosphotungstic acid gives an immediate white precipitate even in a dilution of 1 to 10,000. Phosphomolybdic acid in solutions of the same strength yields a yellow precipitate.

The physiological action of this base has been examined by R. WURTZ, who found the lethal dose for rabbits, etc., to be about one gramme per kilogramme of body weight. It produces stupor, paralysis, which at first appears in the rear extremities; the sensibility becomes diminished and the pupils are dilated and unresponsive to light; the rate of heart-beat is lowered, and the rectal temperature falls as low as 35°; death follows a more or less prolonged coma.

TANRET obtained by the action of ammonia on glucose a number of bases, to which he applied the generic name of glucosines. One of these, having the formula $C_{14}H_{10}N_2$ ($C = 6$), corresponds in its formula and its general properties to MORIN's base $C_7H_{10}N_2$ ($C = 12$), and, in fact, the two bases are considered by TANRET to be identical.

It is interesting to note in this connection that alkaloidal bases have been found in petroleum by BANDROWSKI, and that similar basic substances have been detected by WELLER in paraffin oil.

Most of the solvents in common use, such as alcohol, ether, chloroform, benzole, petroleum ether, amyl alcohol, etc., have been shown at different times to contain basic pyridine compounds, though ordinarily in very minute quantity. On the other hand, HAITINGER has found in some specimens of amyl alcohol as much as 0.5 per cent. of pyridine.

SUSOTOXINE, $C_{10}H_{26}N_2$(?), is a base isolated by NOVY in 1890 from cultures of the hog-cholera bacillus of SALMON (swine-plague of BILLINGS). It is probably identical with the base obtained by v. SCHWEINITZ from the same germ, although the formula ascribed to it by him is $C_{14}H_{32}N_2$. The free base has not been obtained. The hydrochloride forms a light-yellow syrup which shows no tendency to crystallize. It is soluble in water and in absolute alcohol, and is somewhat hygroscopic. When heated with fixed alkali it gives off a strong amine odor, such as is perceived on evaporating the original culture-fluid, if it happens to be alkaline in reaction.

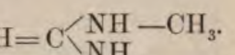
The platinochloride is obtained by precipitation as a light, flesh-colored, granular precipitate. It is readibly soluble in water, from which it can be reprecipitated by addition of absolute alcohol. From aqueous solution, when allowed to evaporate slowly, it crystallizes in long, thick needles.

The mercurochloride is thrown down from solutions of the hydrochloride in absolute alcohol, by alcoholic mercuric chloride, as a heavy, white, granular precipitate. This readily dissolves on the addition of a small quantity of water, and can be perfectly reprecipitated by addition of absolute alcohol. On treatment with hydrogen sulphide it is readily decomposed, yielding the pure hydrochloride

The aurochloride is very soluble in water and alcohol. From the alcoholic solution it may be partially precipitated by ether as a light-yellow, oily precipitate, which is adherent to the sides and bottom of the tube.

Physiological Action.—The base is toxic only in relatively large doses, as seen from the following experiment. About 100 milligrammes, dissolved in a little water, were injected subcutaneously into a young rat. The animal was at first quiet, apparently unwilling to move. After some ineffectual attempts at jumping, it settled down in a recumbent position, and when placed on its side was unable to rise. Respiration was at first retarded, later increased, but toward the end was again very slow. Convulsive tremors shook the body at frequent intervals. The animal kicked vigorously. Reflexes were present almost to the end. As death approached, the red eyes whitened and took on a glazed, opaque appearance. Death resulted in one and a half hours. The animal was on its side, the feet extended. Post-mortem examination showed the heart arrested in diastole, lungs rather pale, stomach contracted, serum in thoracic cavity, subcuta pale and œdematous. Repeated doses of smaller quantities seem to confer a partial immunity to the action of the germ.

METHYL-GUANIDINE, $C_2H_7N_3$,



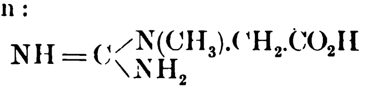
$$=NH=C\left\langle\begin{matrix}NH-CH_3.\\NH_2\end{matrix}\right.$$

This base has long been known as a product of the oxidation of creatine and creatinine, but had never been met with in animal tissues. BRIEGER in 1886 (III., 33) obtained it from horseflesh which was allowed to decompose in a closed vessel at a low temperature (—9° to + 5°) for four months. BOCKLISCH (*Ber.* **20**, 1441) isolated it from impure cultures on beef-broth of FINKLER and PRIOR'S vibrio proteus, containing ordinary putrefaction bacteria, for twenty to thirty days at 37°–38°. Vibrio proteus alone seems incapable of forming this base. The comma bacillus after some time (six weeks) partially decomposes creatinine with formation of a small quantity of methyl-guanidine (BRIEGER). The bacillus of anthrax likewise is capable of transforming creatine into methyl-guanidine.

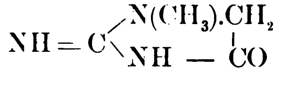
It occurs in the mercuric chloride filtrate (BRIEGER), from which it is obtained, after the removal of the mercury by hydrogen sulphide, by precipitation with phosphomolybdic acid. The precipitate is decomposed with neutral lead acetate, and the filtrate from this, after removal of the lead by hydrogen sulphide, is concentrated and then sodium picrate added. The resinous picrate precipitate is purified by boiling with much water, and, finally, it is recrystallized from boiling absolute alcohol. According to BOCKLISCH, it occurs in the mercuric chloride precipitate (not in the filtrate), from which it is isolated, after removal of the mercury and concentration of the clear filtrate, by precipitation with sodium picrate. The precipitate containing cadaverine, methyl-guanidine, and creatinine, is boiled with absolute alcohol (cadaverine picrate is insoluble) and the alcoholic solution is then evaporated to drive off the alcohol and taken up with water. From this aqueous solution, after removal of picric acid, methyl-guanidine is precipitated by gold chloride, whereas creatinine remains in solution.

This ptomaïne is identical with the synthetic methyl-guanidine (methyluramine) which can be readily obtained by boiling a creatine solution with mercuric oxide or with lead dioxide and dilute sulphuric acid (DESSAIGNES). The parent substance of methyl-guanidine as it occurs in putrefaction is undoubtedly the creatine which exists preformed in the muscular tissue. If such is the case, the bacteria engaged in its production must be considered as possessing an oxidizing action, since this base is prepared synthetically from creatine by oxidation. That creatine does not offer much resistance to the action of bacteria is shown in the fact that FRIEDLÄNDER'S pneumonia coccus, which possesses but small chemical powers, is capable of slowly but steadily decomposing creatine, yielding as one of the products acetic acid. STRECKER and ERLENMEYER, as well as BAUMANN, have shown that creatine, although a substituted guanidine, is not poisonous, but is readily converted into creatinine, which is a relatively toxic substance. On the other hand, guanidine and methyl-guanidine are quite violent poisons. This is, therefore, another instance in which a toxic substance is formed by the action of bacteria from a previously non-poisonous base (see page 244). According to LOSSEN, guanidine is formed, though in small quantity, in the oxidation of albumin.

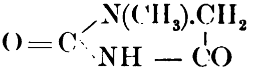
The formulæ of these closely related substances are here given for comparison:

Creatine,


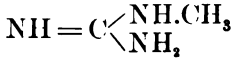
$$NH=C\begin{array}{l}\diagup N(CH_3).CH_2.CO_2H\\ \diagdown NH_2\end{array}$$

Creatinine,


$$NH=C\begin{array}{l}\diagup N(CH_3).CH_2\\ \diagdown NH\ -\ \ CO\end{array}$$

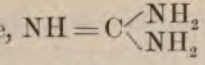
Methyl-hydantoïne,


$$O=C\begin{array}{l}\diagup N(CH_3).CH_2\\ \diagdown NH\ -\ \ CO\end{array}$$

Methyl-guanidine,


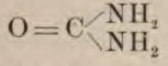
$$NH=C\begin{array}{l}\diagup NH.CH_3\\ \diagdown NH_2\end{array}$$

Guanidine,



$NH=C\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix}$

Urea,



$O=C\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix}$

Methyl-guanidine forms a colorless, easily deliquescent mass possessing a strong alkaline reaction. On heating with potassium hydrate it decomposes, and yields ammonia and methylamine. It is a highly poisonous base.

The HYDROCHLORIDE, $C_2H_7N_3.HCl$, can be obtained from the picrate by dissolving the latter in water acidulated with hydrochloric acid, and extracting the solution with ether to remove the picric acid. The colorless aqueous solution now, on evaporation, yields a thin syrup which crystallizes in vacuum to compact prisms. These are insoluble in alcohol, and give with platinum chloride a double salt of monoclinic needles (HAUSHOFER) which are very easily soluble (1 part in about 7 parts water, TATARINOW).

The AUROCHLORIDE, $C_2H_7N_3.HCl.AuCl_3$ (Au = 47.71 per cent.) forms rhombic crystals (HAUSHOFER) which are easily soluble in ether, more difficultly in water or alcohol; readily soluble (BRIEGER). It readily decomposes on heating in pure water, but may be recrystallized from water acidulated with hydrochloric acid. It melts at 198°.

The PICRATE, $C_2H_7N_3.C_6H_2(NO_2)_3OH$, comes down at first as a resinous precipitate, which when boiled with much water solidifies in the form of felted needles. It is very difficultly soluble in water, and can be purified by repeated recrystallization from boiling absolute alcohol—distinction from cadaverine. It melts at 192°.

The OXALATE, $(C_2H_7N_3)_2.H_2C_2O_4+2H_2O$, forms crystals which are easily soluble in water.

Physiological Action.—Methyl-guanidine as obtained from putrefying flesh is identical in its physiological action with the synthetic base. It has already been stated that the non-poisonous creatine is readily converted into the relatively energetic poison creatinine. The latter substance possesses a paralyzing action differing very much from its decomposition-product methyl-guanidine. This base is very poisonous, and the symptoms are marked by dyspnœa, muscle tremor, and general clonic convulsions. **Brieger** has observed the following symptoms on injection of about 0.2 gramme of methyl-guanidine into a guinea-pig: The respiration at once becomes more rapid, and in a few minutes abundant passage of urine and stool takes place; the pupils dilate rapidly to the maximum and cease to react. The animal is uneasy but motionless, though not exactly paralyzed. Respiration becomes deeper and more labored, the head moves from side to side, the extremities become gradually paralyzed; dyspnœa sets in, the animal falls on its side and dies (twenty minutes) amid general clonic convulsions of short duration. Fibrillary twitchings of the trunk muscles are observed only in the beginning. Post-mortem showed the heart to be stopped in diastole, the intestines filled with fluid, the bladder contracted, the cortex of the kidney hyperæmic, but the papillæ of the kidneys surprisingly pale.

MORRHUINE, $C_{19}H_{27}N_3$, was obtained by **Gautier** and **Mourgues** (1888) from the mother liquors of aselline on concentration of the platinum-containing liquid. This substance constitutes about one-third (0.07 per cent.) of all the bases found in cod-liver oil, and is named from Gadus morrhua, the ordinary codfish. The free base is an oily, very thick, amber-yellow liquid, the odor of which resembles somewhat that of syringa. It floats on water and partially dissolves; is more soluble in ether and in alcohol. The base is very alkaline and is caustic to the tongue. It absorbs carbonic acid and is non-volatile. The salts of copper are precipitated by it, but the hydrate formed is not redissolved

The hydrochloride is very deliquescent. The gold salt forms a yellow precipitate which readily dissolves on warming. The platinum salt, $C_{19}H_{27}N_3.2HCl.PtCl_4$ (Pt = 27.56 per cent.), crystallizes in barbed needles, which are quite soluble. (Separation from aselline, p. 230).

Physiological Action.—The base possesses the property of exciting the appetite; it acts as a diaphoretic and above all as a diuretic. 0.029 gramme given subcutaneously to a guinea-pig produced in two and a half hours a loss of 13.5 grammes in the weight of the animal. The same effect is produced in birds. Strong doses (0.1 gramme per kilogramme) produce fatigue and hebetude.

A BASE, $C_{13}H_{20}N_4$, was obtained as early as 1868 by OSER, who observed its formation during the fermentation of pure cane-sugar by means of yeast. The hydrochloride when dried in vacuo is said to form a white, very hygroscopic foliaceous mass, which soon becomes brown on exposure to air. At first it imparts a burning taste, which is soon replaced by a very bitter sensation.

A BASE corresponding to the formula $C_{17}H_{38}N_4$ was obtained by GAUTIER and ETARD from the mother-liquors of the platinochloride of the base $C_8H_{13}N$. Very little is known, however, in regard to the general properties of this base, owing to the small quantity which could be isolated. This base and the one obtained by OSER from the yeast-fermentation of sugar, $C_{13}H_{20}N_4$, and aselline, $C_{25}H_{32}N_4$, are the only ptomaïnes thus far isolated which are known to contain four atoms of nitrogen.

The PLATINOCHLORIDE, $C_{17}H_{38}N_4.2HCl.PtCl_4$ ($Pt = 27.52$ per cent.), is readily soluble, and crystallizes in needles which possess a light-yellow flesh color. When heated to 100°, it slowly decomposes, giving off a syringa-like odor.

ASELLINE, $C_{25}H_{32}N_4$, isolated by GAUTIER and MOURGUES (1888), together with five other bases from cod-liver oil. (See p. 263.) It is present only in small quantity in the oil. The name is derived from Asellus major, the great codfish. The free base is thrown down from the solutions of the hydrochloride by the addition of alkali, in amorphous white floccules which are almost insoluble in water. It is almost colorless, but on exposure to the air becomes slightly green. It is not hygroscopic, and possesses a density of about 1.05. On heating it melts to a viscid yellowish fluid, possessing an aromatic odor; is non-volatile. Although almost insoluble in water, it imparts to it an alkaline reaction and a bitter taste. It is soluble in ether, more so in alcohol.

The salts are crystallizable, but are partially dissociated by the action of warm water. The hydrochloride forms crossed or entangled needles which are quite bitter. The gold salt is very reducible. The platinochloride, $C_{25}H_{32}N_4.2HCl.PtCl_4$ ($Pt = 24.41$), is orange-yellow in color; soluble in warm waler, insoluble in cold water (separation from morrhuine, p. 228), and is rapidly changed by boiling water. The mercury salt is precipitated in the cold; redissolves on heating, and then, on cooling, recrystallizes.

In large doses it produces fatigue, short and rapid respiration, and stupor. Three milligrammes of the hydrochloride kills a greenfinch in fourteen minutes.

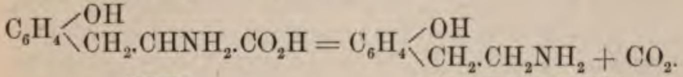
Mydine, $C_8H_{11}NO$, is a non-poisonous base which was obtained by Brieger in 1886 (III., 25) from the putrefaction of about two hundred pounds of human internal organs; and also in cultures of the Eberth bacillus on peptonized blood-serum. It occurs in the mercuric chloride filtrate, and is isolated from it after the removal of the mercury by hydrogen sulphide, by precipitation with phosphomolybdic acid. The gummy precipitate which is produced is decomposed on the water-bath with a solution of neutral lead acetate, and the filtrate on evaporation yields a colorless hydrochloride, crystallizing in plates. It is purified by recrystallization of the picrate.

The free base is strongly alkaline, and possesses an ammoniacal odor. It is characterized by its strong reducing properties. The name mydine is derived from μυδάω, to putrefy. With platinum chloride it gives, after a time, an extremely soluble salt; with gold chloride, a precipitate of metallic gold. On distillation it is decomposed.

The Hydrochloride, $C_8H_{11}NO.HCl$, crystallizes in colorless plates. It gives a blue color with ferric chloride and potassium ferricyanide.

The PICRATE, $C_8H_{11}NO.C_6H_2(NO_2)_3OH$, is obtained in broad prisms, which melt at 195°. It is the only salt suitable for manipulations.

In describing NENCKI's collidine (page 196) it was stated that tyrosin might be looked upon as the source of that base. It would seem, however, to be more appropriately the parent substance of mydine, inasmuch as it decomposes on being heated to 270° into carbonic acid and oxyphenylethylamine, $C_8H_{11}NO$. The change that takes place can be represented by the equation:



$$C_6H_4\begin{cases}OH\\CH_2.CHNH_2.CO_2H\end{cases} = C_6H_4\begin{cases}OH\\CH_2.CH_2NH_2\end{cases} + CO_2.$$

TYROSIN. OXYPHENYL-ETHYLAMINE.

A BASE, $C_5H_{11}NO_2$, was isolated by E. and H. SALKOWSKI (1883) from decomposing fibrin and meat. In its composition it is isomeric with betaine anhydride. It is extremely soluble in water, very difficultly so in alcohol, insoluble in ether, and possesses a semen-like odor and saline taste. The aqueous solution, which is not alkaline in reaction, yields on evaporation a stellate crystalline mass, which on standing over sulphuric acid becomes a white powder, which melts at 156°. It dissolves silver oxide, but not cupric hydrate, thus apparently indicating that it is not an amido acid. Moreover, it does not give a precipitate or blue coloration with copper acetate, or ammoniacal silver nitrate. It thus differed from the then known amido-valerianic acids, its isomers. Recently, however (1891), GABRIEL and ASCHAN showed that δ-amido-valerianic acid agrees with this base in its reactions to copper and silver oxide, copper acetate, and ammoniacal silver nitrate. The gold salt of the synthetic base possessed the same composition as that of SALKOWSKI, and melted at 86°–87°.

The identity of this base with δ-amido-valerianic acid (homopiperidinic acid) would seem to be established, and as such it is regarded. Its structure, then, is represented by

$$NH_2.CH_2.CH_2.CH_2.CH_2.CO_2H.$$

For its synthetic preparation see *Ber.* **24**, 1365 (1891). The base does not seem to possess a toxic action.

The HYDROCHLORIDE, $C_5H_{11}NO_2.HCl$, forms colorless, stellate crystals, which are permanent in the air, and are extremely soluble in water, even in absolute alcohol.

The AUROCHLORIDE, $C_5H_{11}NO_2.HCl.AuCl_3 + H_2O$, is obtained on slow evaporation, as large, well-formed, beautiful dark-yellow crystals. They are probably monoclinic, contain water of crystallization, and melt at below 100°.

The PLATINOCHLORIDE gave on analysis results corresponding to the formula $(C_7H_{15}NO_2.HCl)_2PtCl_4$. This may possibly be due to the presence of some higher homologues of the base $C_5H_{11}NO_2$. It forms fine orange-yellow crystals, which are very difficultly soluble in alcohol, easily so in hot water, from which, on cooling, it crystallizes in beautiful plates.

CHOLINE GROUP.—The following four bases are closely related, and, indeed, starting from choline, the oldest and best-known individual, the remaining bases can be readily prepared from it. Moreover, they can all be prepared synthetically according to methods that will be subsequently indicated. As choline is the most prominent member, we have thought best to class these substances together as constituting the choline group. It is very probable that mydatoxine and mytilotoxine, when their constitution becomes known, will be found to be homologues of certain members of this group.

NEURINE, $C_5H_{13}NO = C_2H_3.N(CH_3)_3.OH$.—This substance was obtained and named thus by LIEBREICH (1865), who prepared it by boiling protagon for twenty-four hours with concentrated baryta. Previous to its discovery as a decomposition-product of protagon from the brain it was prepared synthetically by HOFMANN (1858) by treating trimethylamine and ethylene bromide with potassium hydrate or silver oxide. BAEYER (1866), by boiling an alcoholic extract of the brain with baryta water, obtained on separation by three different methods, a base, or rather a mixture of bases, which, on analysis, gave results corresponding to the three formulæ :

1	2	3
$(C_5H_{14}NOCl)_2PtCl_4$	$(C_5H_{12}NCl)_2PtCl_4$	$(C_5H_{14}NCl)_2PtCl_4$

Formula No. 3 was the one accepted by LIEBREICH for neurine, but, according to BAEYER, LIEBREICH'S neurine salt is not simple, but is a mixture of Nos. 1 and 2. He himself accepts formula No. 1 as the platinochloride of neurine, and distinctly states (*Annal. d. Chem. u. Pharm.*, **142**, 323, 1867) that neurine is in composition trimethyloxyethyl-ammonium hydroxide. And, according to him, choline from bile, and sinkaline from white mustard, appear to be identical with neurine.

This nomenclature of BAEYER'S was at first adopted by WURTZ and others, who showed that the oxyethyl base was identical with choline and sinkaline. On that account STRECKER, in 1868 (*Annal.*, **148**, 79), suggested the restriction of the name choline to the oxyethyl base, and to reserve the name neurine for the base whose platinochloride is represented in No. 3, as originally was done by LIEBREICH. In 1869 LIEBREICH showed conclusively that pure protagon, when heated with baryta for twenty-four hours, yields a substance having the composition of the vinyl base :

$$N(CH_3)_3.C_2H_3.OH.$$

The platinochloride of this base crystallized in five-sided yellow plates, which, after a time, on exposure to the air, became cloudy ; on treatment now with water a portion dissolved, and the solution was found to contain the oxyethyl base. Furthermore, he observed that when the alcoholic extract of the brain, from which all the protagon had been removed, is treated with baryta, only the latter, the oxyethyl base, is obtained. Finally, in 1870, WURTZ abandoned the use of the term neurine to designate the oxyethyl base, and returned to the name choline, originally applied to the oxyethyl base by its discoverer, STRECKER. Nevertheless, the confusion in the use of these two terms continued to exist, and even at the present time it is the cause of no little misunderstanding. Thus, MARINO-ZUCO (1885), in his excellent researches on the genesis of ptomaïnes, applies the term neurine, following BAEYER's precedent, to the oxyethyl base, $C_5H_{15}NO_2$, which is really choline, according to the proper nomenclature.

We have gone somewhat at this point in detail into the history and the proper use of the terms neurine and choline because of the confusion which is sure to arise if the distinction is not thoroughly borne in mind. The name neurine, then, should be used only to denote the vinyl base $C_5H_{13}NO$. It is trimethyl-vinyl-ammonium hydrate. On the other hand, choline is applied to the oxyethyl base $C_5H_{15}NO_2$, which is trimethyl-oxyethyl-ammonium hydrate.

Neurine has been obtained by BRIEGER (1883) in the putrefaction of horse, beef, and human flesh for five to six days in summer. It also occurs in the commercial, so-called "neurine," together with choline (BRIEGER, I., 34). LIEBREICH obtained it in the decomposition of protagon by baryta. And BRIEGER (I., 60) also has isolated it along with choline from fresh human brains, by boiling with baryta; but has not obtained it by digesting the brains on the water-bath with two per cent. hydrochloric acid. It has been found in putrid, and as result of this change poisonous, mushrooms (BERLINERBLAU, 1888).

The genesis of neurine is still rather obscure, and it is to be hoped that future investigations may shed more light upon the mysterious production of this highly poisonous base. Its occurrence in the brain together with choline would seem to indicate that it is either derived from choline by the removal of water, or that it exists together with choline, partly replacing the latter in the molecule of protagon (lecithin), according to the hypothesis put forward by LIPPMANN (page 241). The question of its derivation from choline by withdrawal of a molecule of water has already been subjected to an interesting experimental discussion. CH. GRAM attempted to explain the production of neurine and other muscarine-like ptomaïnes as due to the dehydrating action of the acids employed in the methods of extraction, and, indeed, he claimed to have converted choline platinochloride, by heating with hydrochloric acid, into neurine. This statement has been disputed by BRIEGER, who showed that the platinochloride of choline, as well as the hydrochloride, may be heated with fifteen or thirty per cent., or even concentrated hydrochloric acid, for six to eight hours on a water-bath, without any conversion whatever (III., 15). That neurine may be obtained from choline, at least by chemical processes, was shown by BAEYER, in 1866, who found that choline chloride, when heated with several times its volume of concentrated hydriodic acid and some red phosphorus, gave a compound $C_5H_{13}NI_2$ which, on digestion with fresh, moist silver oxide, yielded a vinyl base identical with that previously obtained synthetically by HOFMANN, and now known as neurine. BRIEGER has tried, unsuccessfully, to bring about this dehydration by the putrefaction of pure choline (I., 59). However, SCHMIDT and WEISS (1887) were more successful, and they found that choline, as well as the hydrochloride and lactate, is changed by the action of microörganisms into the strongly poisonous neurine. Their results are given in full under choline (see page 244.) From what has been said it is evident that neurine can only arise from choline, and this, as will be seen later, is derived from lecithin.

Neurine is almost invariably accompanied by choline, from which, however, it can be readily separated by the difference in the solubilities of the platinochlorides. It occurs in the mercuric chloride precipitate (and in the filtrate), and from this it can be obtained, after removal of the mercury, by precipitating the solution of the mixed hydrochlorides in absolute alcohol by platinum chloride. The platinochlorides are then separated by recrystallization from water, since the neurine is difficultly soluble, while the choline salt is readily soluble.

The free base possesses a strong alkaline reaction, and on contact with the fumes of hydrochloric acid it yields a cloud. According to LIEBREICH, the alkaline solution cannot be neutralized by passing through it carbonic acid.

The CHLORIDE, $C_5H_{12}N.Cl$, is extremely poisonous, and crystallizes in fine hygroscopic needles.

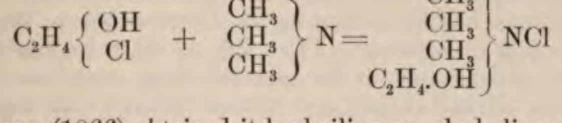
The PLATINOCHLORIDE, $(C_5H_{12}N.Cl)_2PtCl_4$ (Pt = 33.60 per cent.), is difficultly soluble in hot water, and crystallizes in beautiful, well-formed octahedra belonging to the regular system. No twin-crystals are observed. Sometimes the crystals contain water of crystallization, at other times they do not (BRIEGER, I., 33). According to LIEBREICH, it forms from an aqueous solution in five- or six-sided, heaped-up plates resembling urea nitrate, while from an alcoholic solution it forms needles, which on exposure to air become opaque, and are partially converted into the oxyethyl base—choline.

The AUROCHLORIDE, $C_5H_{12}N.Cl.AuCl_3$ (Au = 46.37 per cent.), forms flat prisms, which are difficultly soluble in hot water (BRIEGER.) Dissolves easily, and can be purified by crystallization (LIEBREICH).

Physiological Action.—Neurine is exceedingly poisonous, even in small doses, and in its action it strongly partakes of the characteristic stamp of poisoning by muscarine. The injection of a few milligrammes into frogs produces in a short time a complete paralysis of the extremities, with deadening of reflex excitability. Respiration stops first, while the rate of heart-beat gradually decreases till, finally, stoppage in diastole takes place. The injection of atropine at this point does away with the effect of neurine, so that the heart begins to beat again. Previously atropinized frogs, as a rule, withstand the action of the poison. Immediately after the introduction of this substance there can be observed a distinct period of exaltation, which, however, soon gives way to the characteristic stage of depression seen in the progressive slowing of the rate of heart-beat. Of the warm-blooded animals, cats seem to be much more sensitive to its action than mice, rabbits, or guinea-pigs. The symptoms seen in rabbits are profuse moistening of the nasal cavities and upper lip, which is succeeded by an intensely profuse salivation; later on there is noticeable an abundant secretion from the nasal mucous membrane and from the eyes; the latter, however, ceases in a short time.

The movements of the heart and of respiration are at first quickened and strengthened, but before long the paralytic effects produce a constant slowing and weakening, till finally complete cessation of both movements results. The decided dyspnœa observed gradually alters its character, and just before death the respiration is irregular and superficial. The heart, as in frogs, continues to beat after the respiratory movements have ceased, until finally it stops in diastole. Direct application of concentrated solutions of the poison to the eyes produces almost always a contraction of the pupil, while a similar but less constant contraction is seen when it is injected. The peristaltic action of the intestines is heightened to such an extent that continual evacuation takes place. Just before death, violent clonic convulsions occur. Atropine possesses a strong antagonistic action toward neurine, and the injection of even a small quantity is sufficient to dispel the symptoms just described.

Choline, $C_5H_{15}NO_2 = C_2H_4OH.N(CH_3)_3.OH$.—This base is identical with the sinkaline of von Babo, the bilineurine of Liebreich, and the neurine of Baeyer, Marino-Zuco, and others. According to Schmiedeberg and Harnack, it is identical with Letellier's amanitine (agaricine), to which they assign, however, the formula $(CH_3)_3N.(CHOH.CH_3)OH$. Choline was first prepared, and so named, by Strecker, in 1862, by treating hog-bile with hydrochloric acid. It was prepared synthetically by Wurtz (1868) by direct union of ethylene chlorhydrine and trimethylamine. The reaction that takes place can be represented by the equation:



$$\mathrm{C_2H_4}\begin{cases}\mathrm{OH}\\ \mathrm{Cl}\end{cases} + \left.\begin{matrix}\mathrm{CH_3}\\ \mathrm{CH_3}\\ \mathrm{CH_3}\end{matrix}\right\}\mathrm{N} = \left.\begin{matrix}\mathrm{CH_3}\\ \mathrm{CH_3}\\ \mathrm{CH_3}\\ \mathrm{C_2H_4.OH}\end{matrix}\right\}\mathrm{NCl}$$

Baeyer (1866) obtained it by boiling an alcoholic extract of the brain with baryta water; and Liebreich, in 1869, showed that if the alcoholic extract, from which all the protagon had been removed, be thus treated, only choline is formed, whereas pure protagon, on heating with baryta, yields neurine. It has been obtained from the yelk of eggs; from bile; from fresh brains (BRIEGER); from fresh eggs, blood, lungs, and hearts, and from lecithin (MARINO-ZUCO); from human placenta (BOEHM); from the eye; from commercial neurine (BRIEGER); from fresh as well as decomposing internal organs of the cadaver (BRIEGER, 1885); from herring-brine and decomposing pike, three days in midsummer (BOCKLISCH). It has also been isolated from cultures of vibrio proteus (BOCKLISCH), and of comma bacillus (BRIEGER). EHRENBERG (1887) found it in poisonous sausage, and, by growing a bacillus obtained from this, on liver.

Not only has choline been met with in the animal tissues, but it has also been observed within the last few years to be very widely distributed in the vegetable kingdom, especially so in fatty seeds. Thus, it has been found (HARNACK, 1876) accompanying muscarine, in toadstool (Agaricus muscarius); in hops, and hence in beer (GRIESS and HARROW; in the seeds of Trigonella, in Indian hemp, areca- and earth-nuts, hemp seeds and lentils (JAHNS); in the seeds of white mustard, as a glycoside (VON BABO); in ergot (BRIEGER); in the germs of pumpkins and lupines (SCHULZE, *Zeitschr. f. Physiol. Chem.*, **11**, 365); in beech-nuts and morels (Helvella esculenta, Boletus lusidus, Amanita pantherina, BÖHM); in flores sambuci (elder), and extracts of belladonna, hyoscyamus, ipecacuanha root and Acorus calamus (KUNZ), and Scopolia Japonica (SCHMIDT and HENSCHKE); in the sprouts and cotyledons of Soja beans (SCHULZE, 1888), in the fat from hog's bean, vetch, peas and lupines (JACOBSON, 1889); from the lecithin of lupine seeds (SCHULZE and STEIGER); and in Cheken leaves (Myrtus cheken, WEIFS). According to LIPPMANN (*Ber.* **20**, 3206), it is present, together with betaine, in the molasses from beet-root sugar. Choline (RITTHAUSEN) and betaine (BÖHM) exist together in cotton-seeds; hence, choline occurs in the press-cakes from cotton-seeds (BÖHM). According to SCHULZE, and also RITTHAUSEN, choline occurs with betaine and another base in the seed of the vetch, and in peas with a base resembling betaine. The two bases have also been found together in Scopolia atropoïdes by SIEBERT.

Choline may readily be prepared, after the method of DIAKONOW, from the yelk of eggs. These are extracted with ether, then with alcohol, and the extracts thus obtained evaporated, when the resulting residues are boiled with baryta for one hour. The filtrate, after the removal of the barium by carbonic acid, is evaporated and the residue is abstracted with absolute alcohol. The alcoholic solution is now precipitated with platinum chloride. BRIEGER (II., 55) has presented a method which is much simpler in its details and obviates the use of the expensive platinum chloride. The tissues rich in lecithin, as yelk of egg, brain, etc., are heated with concentrated hydrochloric acid for some hours on the water-bath. The insoluble residue is filtered off, and the filtrate, after neutralization of the excess of free acid with carbonate of sodium, is evaporated. The residue is extracted with alcohol, and the alcoholic solution is precipitated with alcoholic mercuric chloride. The precipitate thus obtained on recrystallization several times from a large quantity of boiling water, yields the pure double salt of choline.

If desirable, it can be made from pure lecithin, best prepared according to GILSON's method. Yelk of eggs is repeatedly shaken up with ether until the latter is colored only a faint yellow; the ether solution then distilled, the residue taken up in petroleum ether and filtered. The filtrate, in a separatory funnel, is well shaken with 75 per cent. alcohol, and this is repeated several times with fresh alcohol. The alcoholic extracts are combined, allowed to stand for some time, then filtered and subjected to distillation to remove traces of petroleum ether. The solution is now set aside in a cool place for several days; the precipitate which forms consists of cholesterine, etc., and a little lecithin. The alcoholic solution is filtered by decantation, then decolored by boiling with bone-black; rapidly evaporated at 50–60° to a syrupy consistency. This residue is extracted with ether, the solution filtered and evaporated. The lecithin thus obtained is almost perfectly pure, but contains traces of cholesterine. To completely purify it, it can be dissolved in as little absolute alcohol as possible, and set aside to reprecipitate in the cold, —5 to 15°.

In regard to the genesis of choline the preponderance of testimony goes to show that it is derived from the decomposition of lecithin, which, according to the researches of DIAKONOW and others, is one of the most widely distributed compounds, occurring in greater or less quantity in all of the animal tissues. Lecithin, which is a complex esther (STRECKER, HUNDESHAGEN, GILSON), decomposes under the action of acids and alkalies into a base (choline) glycerin, phosphoric acid, and fatty acids (stearic, oleic, palmitic, etc.). GILSON has shown that dilute sulphuric acid slowly decomposes lecithin, forming choline, which, after a few days, disappears; on the other hand, sodium hydrate, in even 1 per cent. solution, rapidly decomposes it. This change is undoubtedly accomplished in a similar manner through the agency of bacteria. BRIEGER (II., 17) is inclined to believe that choline exists preformed in the various tissues, inasmuch as he has been unable to obtain it from the brain, which is rich in lecithin, by boiling with 2 per cent. hydrochloric acid. (See SCHULZE, page 242.) Prolonged heating with concentrated hydrochloric acid was necessary in order to obtain any choline from the brain. This result of BRIEGER's is somewhat at variance with that of MARINO-ZUCO (see *Relazione*, etc., pages 29, 30, and 38), who obtained from 25 grammes of lecithin, by the method of STAS, a small quantity of the aurochloride of a base, while from a similar amount he obtained more relevant quantities by the method of DRAGENDORFF.

The occurrence of choline in the vegetable kingdom would be inexplicable to us at present were it not that we now know of the existence of lecithin-like bodies in plants, from the decomposition of which substantially the same products are obtained as from the lecithin obtained from the animal tissues. The existence of such a body in plants was first predicted by SCHEIBLER in 1870, who was led to this conclusion in his celebrated study of beet-root sugar, because of the presence of oleic acid, glycerin, phosphoric acid, and betaine, as well as cholesterin, in the beet-root extracts. This hypothesis was confirmed by HOPPE-SEYLER, who, in 1879, found a lecithin substance in yeast. SCHULZE found a similar compound in the cotyledons of lupine, while JACOBSON observed its presence in mustard-seeds, in fenugreek-seeds, in maize and wheat, in the fat from beans, peas, vetch, and lupines. HECKEL showed its presence in globularia, and LIPPMANN has found it in beet-root. According to HOPPE-SEYLER, this lecithin-like substance exists in all vegetable cells undergoing development. SCHULZE and LIKIERNIK (1891) were the first to prepare lecithin in a pure condition from plants. It was found to possess the same properties and yield the same decomposition-products as lecithin from animal tissues. Up to the present time lecithin has always been supposed to contain a radical, which gives rise to choline on saponification, as an essential component, while on the other hand the fatty acids entering its molecule are well known to be replaceable by one another. Thus we may have a di-stearine lecithin as well as a di-oleine lecithin. The existence of several lecithins in the yelk of eggs has been recognized for some time, and according to SCHULZE and LIKIERNIK this is also true of the lecithins in plants. Recent observations of LIPPMANN (*Ber.* **20**, 3206) show that the above basic radical, hitherto regarded as constant in lecithin, may possibly be capable of replacement by other similar radicals. He found on saponifying with baryta two different specimens of lecithin, both obtained from beet-root, that while one of them yielded oleic acid, glycerin, phosphoric acid, and betaine; the other lecithin gave oleic acid (and some other fatty acids), glycerin, phosphoric acid, and choline, with no betaine—at least not in isolable quantity. This remarkable difference has led LIPPMANN to suggest an explanation which, while it may not be the correct one, nevertheless possesses a high degree of probability. According to him, the lecithin molecule may contain interchangeable basic radicals in the same manner that it contains interchangeable acid radicals. This view is supported not only in the case of beet-root, where choline and betaine exist together, but the same two bases have been observed in cotton-seeds. A similar coexistence was observed in the toad-stool (Agaricus muscarius), in which choline and muscarine were found. And, lastly, the same condition holds true probably for mytilotoxine and betaine, which were shown to be present together in poisonous mussels.

Lecithin cannot always be regarded as the source of choline in plants, since this base is known to occur as a glucoside in the seeds of white mustard. The sinapin decomposes according to the equation :

$$\underset{\text{Sinapin.}}{C_{16}H_{23}NO_5} + 2H_2O = \underset{\text{Choline.}}{C_5H_{15}NO_2} + \underset{\text{Sinapic Acid.}}{C_{11}H_{12}O_5}.$$

According to Schulze (1891) the choline which is isolated from pea- and vetch-seeds exists preformed in the seeds, and does not result from lecithin by the process of extraction. This is also probably true with reference to cottonseed-cake. The condition in which betaine exists is not determined.

The protoplasm itself is another possible source of choline as well as of other nitrogenous bases, as xanthine, etc. We know from Drechsel's brilliant investigation (1890) that casein on treatment with hydrochloric acid and stannous chloride yields ammonia, amido acids, and organic bases—*lysatine*, $C_6H_{13}N_3O_2$, and *lysatinine*, $C_6H_{11}N_3O$ — homologues of creatine, $C_4H_9N_3O_2$, and creatinine, $C_4H_7N_3O$. From lysatinine urea can be readily obtained by treatment with baryta. Subsequently, Siegfried (1891) showed that vegetable protoplasm (conglutin from lupine) when treated in the same way yields similar products. Later, Schulze demonstrated that the base, *arginine*, $C_6H_{14}N_4O_2$, is formed in lupine sprouts at the expense of the proteids present, and he pointed out that this base is probably related to lysatine, from which it differs only by NH (see next chapter).

DECOMPOSITIONS OF CHOLINE.—BAEYER (1866) succeeded in converting choline into neurine by a purely chemical process. This was accomplished by heating choline chloride with concentrated hydriodic acid and red phosphorus in a sealed tube at 120°–150°, whereby the compound $C_5H_{13}NI_2$ was formed. The iod-iodide of choline thus obtained, on treatment with moist silver oxide, gave a base whose platinochloride corresponded to the formula $(C_5H_{12}NCl)_2PtCl_4+H_2O$. This double salt, according to BAEYER, is readily soluble in water, and gives reactions similar to choline. Although BAEYER is emphatic in his assertion that this is the vinyl compound (neurine) formed from the oxy-ethyl base (choline), yet it seems that there is room for doubt in regard to the interpretation of his results. Thus neurine platinochloride is difficultly soluble in water, contrary to the behavior of the platinochloride obtained by him. On the other hand, choline platinochloride is easily soluble in water, and it would seem, therefore, that BAEYER has not converted choline into neurine, but rather has regenerated choline from its iod-iodide. If such were the case, we would expect that the iod-iodide of neurine, $C_5H_{13}NI_2$, which has the same composition as the corresponding derivative of choline, would yield, on treatment with silver oxide, the oxy-ethyl base. BAEYER has apparently not been able to effect this change, since he holds that the vinyl base may be prepared from the oxy-ethyl, but that the reverse, the preparation of the oxy-ethyl base from the vinyl compound, cannot be accomplished.

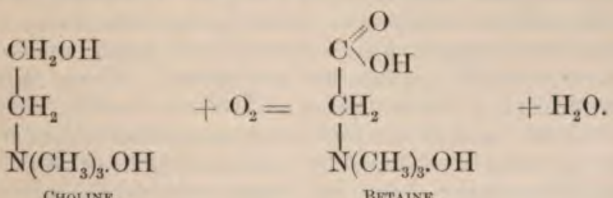
Whether the change described by BAEYER takes place or not, it is, nevertheless, certain that choline does not readily give up a molecule of water and thus become converted into neurine. CH. GRAM announced, in 1886, that choline chloride and lactate on heating on the water-bath decompose, and that this conversion into the vinyl base was complete when the aqueous hydrochloric acid solution of choline platinochloride was heated for five or six hours on the water-bath. In this way GRAM endeavored to explain the formation as due to the action of acids upon choline, but BRIEGER has shown that the platinum salt of choline, as well as its hydrochloride, can be heated with fifteen or thirty per cent., or even concentrated, hydrochloric acid for six or eight hours without undergoing any change into neurine, thus disproving the results obtained by GRAM. E. SCHMIDT has confirmed BRIEGER'S observations in regard to the resistance of choline to decomposition by acids, but he has gone further, and has shown that what the action of acids has failed to do is readily accomplished through the agency of bacteria. He found that choline chloride, when allowed to stand with hay infusion, or with dilute blood for fourteen days at 30°–35°, it almost entirely decomposed, yielding large quantities of trimethylamine and a base, the platinochloride of which resembles in form and solubility the double salt of neurine, and possesses a similar physiological action. Choline lactate in hay infusion developed an odor of trimethylamine in twelve hours, but at the end of fourteen days a good deal of choline was still present. In this case no neurine was present, but instead a homologous base was found, which can be obtained synthetically by the action of trimethylamine on allyl bromide. According to MEYER, of Marburg, this base does not possess the muscarine-like action of neurine, but resembles more closely pilocarpine.

BRIEGER (I., 59) had unsuccessfully tried to transform choline into neurine by putrefaction. He observed that the choline decomposed with extreme slowness, even when the putrefaction was carried on at a higher temperature, yielding only trimethylamine. WURTZ (1868) showed that dilute solutions of free choline can be heated to boiling without any perceptible decomposition. Concentrated solutions, however, decompose with the formation of trimethylamine and glycol, $C_2H_4(OH)_2$ (see page 190). The decomposition of choline was studied somewhat by MAUTHNER (1873), who confirmed WURTZ'S observation that choline was scarcely decomposed by boiling water, and he showed that when exposed to the action of decomposing blood it yielded trimethylamine. The results obtained by K. HASEBROEK (*Zeitschrift f. Physiol. Chem.*, **12**, 151, 1888) deserve special mention at this place. He carried on the putrefaction of very dilute solutions of the chloride of choline in the presence of little or no oxygen in HOPPE-SEYLER fermentation flasks. Sewer slime, because of its strong fermentative properties, was used to induce the putrefaction, and calcium carbonate was added to neutralize any acidity that might develop during the fermentation.

The fermentation, as shown by the evolution of gases, lasted for about three months. The total quantity of gas given off was about one litre from 1.17 grammes choline chloride. The gases consisted almost entirely of carbonic acid and marsh gas. No hydrogen was evolved. When the fermentation ceased the flask was opened and several cubic centimetres of the almost neutral clear liquid were injected under the skin of a rabbit without producing the least effect.

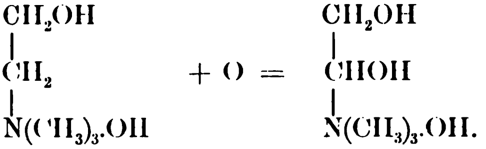
This liquid distilled with alkali gave methylamine and ammonia. What is remarkable about this experiment was the total absence of the higher amines—as, for instance, trimethylamine, which has been observed so many times as a decomposition-product of choline. The absence of any poisonous base, as neurine, was probably largely connected with the absence of oxygen.

Free choline ordinarily forms a strongly alkaline syrup which combines readily with acids to form salts, most of which are deliquescent. By oxidation it is converted into betaine (see page 249), and on treatment with concentrated nitric acid it gives rise to muscarine (see page 251). These reactions can be represented by the equations :



$$\begin{array}{l} CH_2OH \\ | \\ CH_2 \\ | \\ N(CH_3)_3.OH \end{array} + O_2 = \begin{array}{l} C\begin{array}{l}\diagup\!\!\!\!\diagup O \\ \diagdown OH\end{array} \\ | \\ CH_2 \\ | \\ N(CH_3)_3.OH \end{array} + H_2O.$$

CHOLINE. BETAINE.



$$\begin{array}{lcl} CH_2OH & & CH_2OH \\ | & & | \\ CH_2 & + O = & CHOH \\ | & & | \\ N(CH_3)_3.OH & & N(CH_3)_3.OH. \end{array}$$

MUSCARINE.

By the action of dilute nitric acid choline is converted into a base the platinochloride of which is efflorescent and corresponds to the formula $(C_4H_{10}N_2O_3Cl)PtCl_4 + 2H_2O$ (SCHMIEDEBERG and HARNACK).

According to MAUTHNER, choline resembles the caustic alkalies in its action. Although putrefying blood decomposes it into trimethylamine, yet, when present in the proportion of 1.4 per cent., it is said to arrest putrefaction. A 1 to 2 per cent. solution is said to dissolve fibrin or coagulated albumin on boiling.

The free base, as well as the carbonate, is dimorphous and forms thin plates or long needles.

The CHLORIDE, $C_5H_{14}NO.Cl$, is easily soluble in water and in absolute alcohol (separation from neuridine hydrochloride). It crystallizes over sulphuric acid to needles which readily deliquesce in the air.

The PLATINOCHLORIDE, $(C_5H_{14}NO.Cl)_2PtCl_4$ ($Pt = 31.64$ per cent.), presents an interesting case of trimorphism. It crystallizes in monoclinic plates (RINNE) which are easily soluble in water, insoluble in alcohol; also in characteristic superposed plates, sometimes in the form of orange-red flat prisms (BRIEGER). From a warm saturated solution containing 15 per cent. alcohol it crystallizes in yellow regular octahedra containing one molecule of water of crystallization (JAHNS); from aqueous solution on slow evaporation it forms plates, clinorhombic prisms, or needles (HOPPE-SEYLER) which are anhydrous. When rapidly crystallized it forms prisms (HUNDESHAGEN, JAHNS, SCHULZE); and if the solution is concentrated the prisms are very thin, almost needles. According to SCHULZE, it sometimes forms beautiful orange-red, chiefly six-sided plates. JAHNS maintains that the plates and prisms belong to the same system; while HUNDESHAGEN holds that they are distinct. Instead of the salt presenting an instance of trimorphism as first stated by HUNDESHAGEN, it would seem that but two forms occur—anhydrous monoclinic and octahedra with one molecule of water of crystallization. It contains always more or less water of crystallization which it does not give up completely over sulphuric acid, but only at 110° (BRIEGER). The natural platinochloride becomes strongly electric on rubbing, whereas the synthetic choline double salt does not become electric. It melts at 225° with effervescence (JAHNS).

The AUROCHLORIDE, $C_5H_{14}NO.Cl.AuCl_3$ ($Au = 44.48$ per cent.), is crystalline and is difficultly soluble in cold water, but can be recrystallized from hot water or from boiling alcohol. It forms prisms, or gold-yellow long needles, which are very easily soluble in hot water and alcohol (LIPPMANN). It can be separated from neuridine aurochloride by its solubility in water (BRIEGER). On heating, the gold salt melts to a brown liquid (SCHULZE) and decomposes at 264°.

The MERCUROCHLORIDE, $C_5H_{14}NO.Cl.6HgCl_2$, is extremely difficulty soluble even in hot water. On this account the mercury salt is very convenient for the separation of choline from accompanying bases.

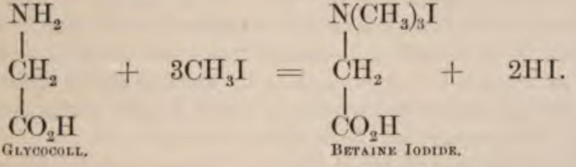
The PICRATE, $C_5H_{14}NO.OC_6H_2(NO_2)_3$, forms long, broad needles which are more easily soluble than neuridine picrate, and hence can be separated by recrystallization. It is more easily soluble in alcohol than in water.

Physiological Action of Choline.—Choline was regarded for a long time as physiologically inert, but this belief was set aside by GAEHTGENS (1870), who showed that, when given in large quantity, it possessed a toxic action. This observation of GAEHTGENS has since been confirmed by GLAUSE and LUCHSINGER, BRIEGER, and BOEHM. The chloride of choline produces in animals the same muscarine-like symptoms of poisoning as are developed by the vinyl base neurine, the only difference lies in the intensity of the action. In order to bring about a physiological disturbance, choline must be given in relatively large doses. Thus, BRIEGER found it necessary to give about 0.1 gramme of choline chloride hypodermically to a one kilogramme rabbit in order to bring out the same effects as are obtained by the injection of 0.005 gramme of the neurine salt. He also found that the fatal dose for a one-kilogramme rabbit was about 0.5 gramme, which is about ten times as large as the fatal dose of neurine chloride. BOEHM observed that doses of 0.025–0.1 gramme produced in frogs general paralysis, which, in a short time, leads to death or recovery; and that in its curara-like paralyzing action, choline resembles artificial muscarine, although the latter is about five hundred times stronger. Atropine, as in the case of neurine and muscarine, antagonizes the action of choline. Thus, 0.05 gramme of the chloride produced in a frog in one hour diastolic stoppage of the heart. This condition was removed by the injection of 0 001 gramme of atropine, the heart-beat rising to the normal in about fourteen minutes; 0.05 gramme of choline chloride, given subcutaneously to a rabbit (1250 grammes) produced salivation, which lasted but a short time, and did not affect the heart-beat and respiration; 0.10 gramme was necessary to bring out all the symptoms; 0.05 gramme, given to guinea-pigs, had no effect whatever.

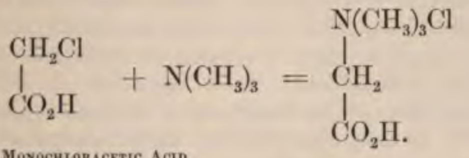
BETAINE (OXYNEURINE), $C_5H_{13}NO_3$.—This base has been well known for some time, because of its occurrence in the vegetable kingdom. Thus, it is present in cotton-seed (BOEHM, RITTHAUSEN and WEGER); in beet-root juice (Beta vulgaris), and hence in beet-root molasses (SCHEIBLER, 1866). It occurs also in cattle-turnip and Lycium barbarum; and is found with choline and another base in vetch-seeds; in peas a base similar to betaine exists (SCHULZE). With choline it occurs in Scopolia atropoïdes (SIEBERT). It does not exist in these substances as such, but is formed from a more complex substance by the action of hydrochloric acid or baryta (LIEBREICH). In this respect it resembles choline, neurine, and probably muscarine. Quite recently, LIPPMANN (1887) has obtained a lecithin-like body from sugar-beet, which, on heating with baryta gave oleic acid, glycerin, and phosphoric acid (glycerin-phosphoric acid), and betaine. Betaine, however, does not seem to be a constant constituent, inasmuch as on one occasion he obtained chiefly choline, and little or no betaine. These two bases also occur together in cotton-seed, and this fact has led **Scheibler** to the conclusion that it is no mere chance. Lecithin, as is well known, may contain variable acid constituents (oleic, stearic, palmitic, etc.), and reasoning on this fact, and on the results of his experiments, **Lippmann** has been led to suppose that it may also contain different bases in variable proportions.

It has been obtained from human urine (**Liebreich**, 1869), and from poisonous and non-poisonous mussel, but not from putrid mussel (**Brieger**, 1885, III., 76). The method for its separation from mussel is described on page 255.

Betaine may be obtained synthetically in several ways: (1) by oxidation of choline with potassium permanganate; (2) by the action of methyl iodide on glycocoll; (3) by treating monochloracetic acid with trimethylamine. The last two methods are of value as indicating the constitution of betaine, and the changes which take place can be represented by the equations:

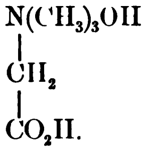


$$\begin{array}{c}\mathrm{NH_2}\\|\\\mathrm{CH_2}\\|\\\mathrm{CO_2H}\end{array} + \mathrm{3CH_3I} = \begin{array}{c}\mathrm{N(CH_3)_3I}\\|\\\mathrm{CH_2}\\|\\\mathrm{CO_2H}\end{array} + \mathrm{2HI.}$$



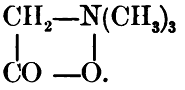
$$\begin{array}{c}\mathrm{CH_2Cl}\\|\\\mathrm{CO_2H}\end{array} + \mathrm{N(CH_3)_3} = \begin{array}{c}\mathrm{N(CH_3)_3Cl}\\|\\\mathrm{CH_2}\\|\\\mathrm{CO_2H.}\end{array}$$

From the formulæ of the salts of betaine it is evident that betaine has properly the composition $C_5H_{13}NO_3$, which is expressed by the structural formula:



$$\begin{array}{l} N(CH_3)_3OH \\ | \\ CH_2 \\ | \\ CO_2H. \end{array}$$

The free base is, however, readily converted into the anhydride, $C_5H_{11}NO_2$, trimethyl glycocoll; the structural formula of which is:



$$\begin{array}{ll} CH_2- & N(CH_3)_3 \\ | & | \\ CO\ - & O. \end{array}$$

Betaine is ordinarily regarded as crystallizing with one molecule of water, and the composition is expressed by the formula: $C_5H_{11}NO_2+H_2O$ (= $OH.N(CH_3)_3.CH_2.CO_2H$). It loses this water of crystallization by heating at 100°, or on standing over sulphuric acid, forming an anhydride of the formula already given. LIEBREICH claims that free betaine possesses the formula $C_5H_{11}NO_2$, because it yields a compound having the composition $(C_5H_{11}NO_2)ZnCl_2$. The free base separates from alcohol in large crystals which deliquesce on exposure to the air. As obtained by BRIEGER from the hydrochloride by treatment with moist silver oxide, it possessed a sweetish taste and neutral reaction. When distilled with potassium hydrate, it yields trimethylamine and other bases, among which a base of the formula $C_8H_{17}NO_5$ occurs in the largest quantity.

The CHLORIDE, $C_5H_{12}NO_2.Cl$, forms beautiful crystals, monoclinic plates, which are permanent in the air, and this can be made use of to effect a separation from the choline salt, which is deliquescent. It is insoluble in absolute alcohol. This fact can be made use of in their separation (LIPPMANN). It can, moreover, be easily separated from other bases by its aurochloride, which is easily soluble. If a little potassio-mercuric iodide is added to a solution of the chloride, there forms a light-yellow or whitish oily precipitate, which is soluble in excess, but on rubbing the sides of the tube with a glass rod it reappears as yellow needles. This is said to be a characteric test (BRIEGER, SCHULZE, 1891).

The AUROCHLORIDE, $C_5H_{12}NO_2.Cl.AuCl_3$ (Au = 43.12 per cent.), forms magnificent cholesterin-like plates, and is easily soluble (BRIEGER). The aurochloride from sugar-beet is said to crystallize in needles or plates, and to be difficultly soluble in cold water (SCHEIBLER, LIPPMANN). The double salt of the ptomaïne melts at 209°, and in this it coincides with that obtained from beet-sugar, as well as with that of the synthetically prepared base (BRIEGER). The platinochloride is yellow and crystalline.

Betaine is not poisonous.

MUSCARINE, $C_5H_{15}NO_3 = C_5H_{13}NO_2 + H_2O$, the well-known toxic principle which SCHMIEDEBERG obtained from poisonous mushroom (Agaricus muscarius), has been obtained also by BRIEGER in 1885 (I., 48) from haddock which had been allowed to decompose for five days. The process by which its isolation was effected is described on page 258. This base is specially interesting, because of the relation it bears to choline, for SCHMIEDEBERG has shown that it is formed when choline, or, better still, the platinochloride, is oxidized by concentrated nitric acid. It is barely possible that BRIEGER'S base is distinct from SCHMIEDEBERG'S; nevertheless, it closely resembles it and apparently is identical.

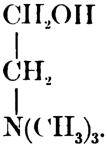
The CHLORIDE, $C_5H_{14}NO_2.Cl$, is obtained on the decomposition of the platinochloride with hydrogen sulphide, as a syrupy residue, which, under the desiccator, shows a tendency to gradually crystallize. It is deliquescent (HARNACK).

The PLATINOCHLORIDE, $(C_5H_{14}NO_2.Cl)_2PtCl_4$ (Pt = 30.08 per cent.), forms as a crystalline deposit of octahedra, which are difficultly soluble in water. They lose their water of crystallization ($2H_2O$) only by means of strong heating.

The AUROCHLORIDE, $C_5H_{14}NO_2.Cl.AuCl_3$, crystallizes in needles, and is difficultly soluble in water, more so than the choline double salt (Harnack).

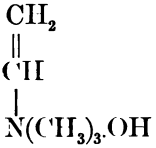
Physiological Action.—Small doses of this ptomaïne induce in frogs total paralysis, with stoppage of the heart in diastole, and this action is antagonized by subsequent injection of atropine, as well as in the case of previously atropinized frogs. Very small doses produce in rabbits profuse salivation and lachrymation, contraction of the pupil, profuse diarrhœa, and passage of urine and semen; finally, the animal dies in convulsions, which, however, are only of short duration.

Constitution of the Members of the Choline Group.—The structure of choline was clearly demonstrated by Wurtz, who accomplished the synthesis of this base by treatment of ethylene chlorhydrine with trimethylamine. This same method can be applied to the synthesis of betaine and neurine by using monochloracetic acid and vinylbromide instead of ethylene chlorhydrine. The structural formulæ which can be deduced from these reactions are as follows:



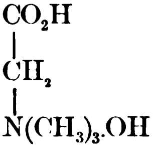
$$\begin{array}{l} CH_2OH \\ | \\ CH_2 \\ | \\ N(CH_3)_3.OH \end{array}$$

Choline.



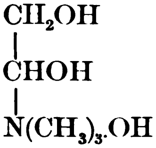
$$\begin{array}{l} CH_2 \\ \| \\ CH \\ | \\ N(CH_3)_3.OH \end{array}$$

Neurine.



$$\begin{array}{l} CO_2H \\ | \\ CH_2 \\ | \\ N(CH_3)_3.OH \end{array}$$

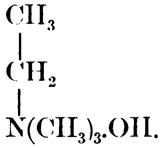
Betaine.



$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ N(CH_3)_3.OH \end{array}$$

Muscarine.

The formulæ of betaine and muscarine are ordinarily given as the anhydrides, but there can be no doubt that the free bases possess the structure indicated above. All these bases, since they can be prepared from choline, may also be considered as oxidation-products of trimethyl-ethyl-ammonium hydrate:



$$\begin{array}{l} CH_3 \\ | \\ CH_2 \\ | \\ N(CH_3)_3.OH. \end{array}$$

MYDATOXINE, $C_6H_{13}NO_2$.—This base was obtained by BRIEGER in 1886 (III., 25, 32) from several hundred pounds of human internal organs which were allowed to stand in closed but spacious wooden barrels for four months, at a temperature varying from —9° to +5°. He obtained much larger quantities of it, however, from horseflesh which had putrefied under the same conditions. In the process of extraction it is found in the mercuric chloride precipitate together with cadaverine, putrescine, and another base, $C_7H_{17}NO_2$. It can be isolated from this mixture by recrystallizing the mercury salts, which removes the cadaverine, because of its difficult solubility in water, and decomposing the soluble mercury salts by hydrogen sulphide. The filtrate freed from mercury is now evaporated to dryness and the residue repeatedly extracted with absolute alcohol, in order to remove putrescine hydrochloride, which is insoluble. The alcoholic solution, after standing some time to permit complete separation of any dissolved putrescine, is then evaporated to dryness and taken up with water. This solution gives, on the addition of gold chloride, a precipitate of the aurochloride of the base $C_7H_{17}NO_2$. The filtrate from this precipitate, containing the mydatoxine, is treated with hydrogen sulphide to remove the gold, and then evaporated to dryness. The colorless, syrupy hydrochloride thus obtained forms with platinum chloride a double salt which is readily soluble in water, and can be purified by repeated recrystallizations from absolute alcohol containing some hydrochloric acid.

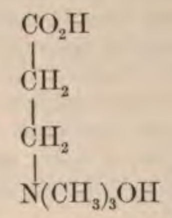
The name mydatoxine is derived from μυδάω, to putrefy. The free base is obtained from the hydrochloride by treatment with moist, freshly precipitated silver oxide, as a strongly alkaline syrup, which solidifies in vacuo to plates. It is insoluble in alcohol, ether, etc. It does not distil without decomposition. It is isomeric with the base, $C_6H_{13}NO_2$, obtained by BRIEGER in 1888 from tetanus cultures.

The HYDROCHLORIDE, $C_6H_{13}NO_2.HCl$, is a colorless, deliquescent syrup which does not form any double salt with gold chloride. With platinum chloride it gives an easily soluble salt. Otherwise it combines only with phosphomolybdic acid, with which it forms cubes. Ferric chloride and potassium ferricyanide yield, after a time, Berlin-blue. It is readily soluble in alcohol.

The PLATINOCHLORIDE, $(C_6H_{13}NO_2.HCl)_2PtCl_4$ (Pt = 29.00 per cent.), melts at 193°, with decomposition. It crystallizes in plates which are extremely soluble in water. It can be readily recrystallized from absolute alcohol acidulated with hydrochloric acid. The mercury salt is readily soluble in water.

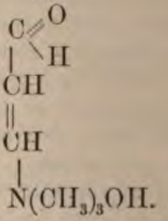
The exact formula of this base, of mytilotoxine, and some other bases, cannot be considered to be permanently settled, inasmuch as the formula of the hydrochloride, $C_6H_{13}NO_2.HCl$, as deduced from the analysis of the platinum double salt, may equally apply to the base $C_6H_{14}NO_2.OH$ as to the base $C_6H_{13}NO_2$. If the first formula is correct, then mydatoxine is a homologue of betaine, and its structure would be expressed by (1).

(1)



$$\begin{array}{l} CO_2H \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ N(CH_3)_3OH \end{array}$$

(2)



The second formula would seem to correspond to an unsaturated aldehyde of the choline group and its structure may be indicated by (2).

This ptomaïne, although it possesses toxic properties, is not, however, a strong poison. Its action is the same as that of the base $C_7H_{17}NO_2$ (see page 262), with which it is associated, except that the symptoms of poisoning develop slower, so that the death of a guinea-pig does not take place for about twelve hours. White mice are very susceptible to the action of these two poisons. A short time after the injection of even small doses they are taken with convulsions which come on in paroxysms. The eyeballs roll upward. Lachrymation, diarrhœa, and dyspnœa come on, and the mice die within a short time.

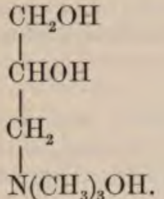
A BASE (?), $C_6H_{13}NO_2$, an isomer of the preceding, was obtained by BRIEGER in 1888 from tetanus cultures. It is not poisonous—distinction from mydatoxine. It probably is an amido-acid. The platinochloride crystallizes in plates, is easily soluble in water and in alcohol, and melts at 197° with decomposition (see page 267).

MYTILOTOXINE, $C_6H_{15}NO_2$, is the specific poison of toxic mussel (Mytilus edulis), from which it was obtained by BRIEGER in 1885 (III., 76). This poison is formed during the life of the animal under certain conditions which have been thoroughly studied by SCHMIDTMANN, VIRCHOW, and others (see p. 40). BRIEGER obtained the poison by extracting toxic mussel with acidulous water, and evaporating this solution to a syrupy consistency. The residue was thoroughly extracted with alcohol, and this solution was treated with lead acetate, in order to remove mucilaginous substances. The filtrate was then evaporated, and the residue extracted with alcohol. Any lead that had dissolved was removed by hydrogen sulphide. The alcohol was expelled, and the resulting syrup was taken up with water and decolored by boiling with animal charcoal. The clear solution was now neutralized with sodium carbonate, acidulated with nitric acid, and precipitated with phosphomolybdic acid. The precipitate was decomposed by warming with neutral lead acetate, and the resulting filtrate, after the removal of the lead by hydrogen sulphide, was acidulated with hydrochloric acid and evaporated to dryness. The residue was extracted with absolute alcohol, whereby betaine, on account of its insolubility, is removed, and the alcoholic solution was precipitated by alcoholic mercuric chloride. The mercury precipitate is repeatedly recrystallized from water, and the poison is obtained as an easily soluble double salt.

The free base as obtained by the addition of alkali to the hydrochloride possesses a disagreeable odor which disappears on exposure to air, and the substance ceases to possess poisonous properties. BRIEGER has proposed the application of this test for the recognition of poisonous mussel; on treatment of these with alkali the characteristic odor is developed. Mytilotoxine is also destroyed on distillation with potassium hydrate and in the distillate there is found an aromatic non-poisonous product and trimethylamine. The free base, therefore, does not exist by itself for any length of time, but soon becomes converted into an inert substance. H. SALKOWSKI has also shown that it is destroyed on boiling with potassium carbonate, whereas its hydrochloric acid solution can be evaporated to dryness and heated to 110° without destroying its poisonous property.

The HYDROCHLORIDE, $C_6H_{15}NO_2.HCl$, prepared from the aurochloride, crystallizes in tetrahedra. It is extremely poisonous and according to BRIEGER produces exactly the same symptoms which have been observed by SCHMIDTMANN in persons who have partaken of poisonous mussels (see page 38). On standing, however, the pure hydrochloride gradually becomes dark and decomposes with loss of its poisonous property—a change corresponding to that which tetanine undergoes (p. 267). The gold salt is better adapted for preservation. The ordinary alkaloidal reagents produce in its solutions, if at all, only oily precipitates.

As stated under mydatoxine, the formula of the hydrochloride, $C_6H_{15}NO_2.HCl$, is applicable to either one of two bases, $C_6H_{16}NO_2.OH$ or $C_6H_{15}NO_2$. The base corresponding to the first formula is evidently a homologue of muscarine, and should possess a similar physiological action. As a matter of fact, mytilotoxine does resemble muscarine somewhat in its action, and its occurrence together with betaine would seem to make it a decomposition-product of lecithin, in which case this base must be looked upon as a member of the choline group. It is interesting to know that a compound corresponding to the formula $C_6H_{16}NO_2.OH$ has been known for some time, and was prepared by HANRIOT in a manner analogous to WURTZ's synthesis of choline, by treating glycerin monochlorhydrine with trimethylamine. This base, trimethyl-glyceryl-ammonium hydrate, has this structure:

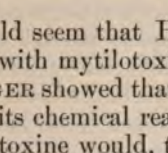


$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2 \\ | \\ N(CH_3)_3OH. \end{array}$$

It would seem that HANRIOT's base might possibly be identical with mytilotoxine, but a careful comparison made by BRIEGER showed that it possesses no physiological action and that its chemical reactions are entirely different.

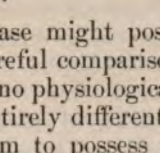
Mytilotoxine would, therefore, seem to possess the formula, $C_6H_{15}NO_2$, as originally given it by BRIEGER. From the fact that on distillation with potassium hydrate it yields trimethylamine, it follows that mytilotoxine is a quarternary base. He is inclined to regard it as a methyl derivative of betaine, which is so common in mussels, and represents it by formula No. 1.

(1)



$$\begin{array}{l} CO_2H \\ | \\ CH.CH_3 \\ | \\ N(CH_3)_3.OH \end{array}$$

(2)



$$\begin{array}{l} CH_2OH \\ | \\ CH.CH_3 \\ | \\ N(CH_3)_3.OH \end{array}$$

No. 1, however, is $C_6H_{15}NO_3$, instead of $C_6H_{15}NO_2$, as above. The formula No. 2, $C_6H_{17}NO_2$, would represent a derivative of choline or muscarine, with only a slightly higher percentage of hydrogen.

The AUROCHLORIDE, $C_6H_{15}NO_2.HCl.AuCl_3 (Au = 41.66$ per cent.), crystallizes in cubes. Its melting-point is 182°.

It is well to observe that BRIEGER has been unable to obtain this base from mussels that were allowed to putrefy for sixteen days.

Physiological Action.—According to BRIEGER, mytilotoxine produces all the characteristic effects seen in mussel poisoning, and it is, therefore, a strong paralysis-producing poison, and resembles curara in its action. This action is explainable now that GLAUSE and LUCHSINGER have shown that all trimethyl-ammonium bases have a muscarine-like action. For the symptoms induced by poisonous mussel see page 38.

GADININE, $C_7H_{17}NO_2$, was found in haddock (1885) which was allowed to decompose in open iron vessels for five days during summer. BRIEGER has also obtained it from cultures of the bacteria of human feces on gelatin. The decomposing mass was thoroughly stirred every day in order to bring it into contact with atmospheric oxygen (BRIEGER, I., 49). It was then treated with water, and hydrochloric acid was added to acid reaction, and after being warmed the mixture was filtered and the filtrate concentrated on the water-bath to a syrupy consistency. This syrupy residue was extracted with water, and the aqueous solution was precipitated with a solution of mercuric chloride. The mercuric chloride precipitate contained a base, the quantity of which, however, was insufficient for a complete analysis (see page 272). The mercuric chloride filtrate, after the removal of the mercury by hydrogen sulphide, was evaporated to a syrup, and this was then repeatedly extracted with alcohol. The alcoholic solution thus obtained contained neuridine, a base of the same composition as ethylenediamine, muscarine, gadinine, and triethylamine. These bases were separated in the following manner: The alcoholic solution gave with platinum chloride a precipitate of neuridine. The filtrate from this platinum precipitate was heated on the water-bath to expel the alcohol, and then the platinum was removed by hydrogen sulphide. The aqueous filtrate was concentrated to a small volume which, on addition of platinum chloride, gave a precipitate of the isomer of ethylenediamine. The mother-liquor from this precipitate was concentrated on a water-bath, and on cooling the platinochloride of muscarine crystallized out. From the mother-liquor of this precipitate on standing in a desiccator, the gadinine double salt crystallized. The mother-liquor from the gadinine platinochloride was treated with hydrogen sulphide to remove the platinum, and the aqueous filtrate on distillation with potassium hydrate gave triethylamine.

Gadinine (from Gadus callarias, haddock) in small doses does not appear to be poisonous; larger doses (0.5–1 gramme) are decidedly toxic and may kill guinea-pigs. The formula of the free base as deduced from the analysis of the platinochloride may be either $C_7H_{17}NO_2$ or $C_7H_{18}NO_2\ OH$.

The HYDROCHLORIDE, $C_7H_{17}NO_2.HCl$, as obtained by the decomposition of the platinochloride with hydrogen sulphide, crystallizes under the desiccator in thick, colorless needles, which are easily soluble in water; insoluble in alcohol. It forms no combination with gold chloride, but does give crystalline precipitates with phosphomolybdic acid, phosphotungstic acid, and picric acid.

The PLATINOCHLORIDE, $(C_7H_{17}NO_2.HCl)_2PtCl_4$ ($Pt = 27.68$ per cent.), is at first quite soluble, and on standing over a desiccator it crystallizes in golden-yellow plates, which, when once formed, are again difficultly soluble in water. It can be recrystallized from hot water. It melts at 214°.

TYPHOTOXINE, $C_7H_{17}NO_2$.—This base was named thus by BRIEGER in 1885 (III., 86), and is regarded by him as the specific toxic product of the activity of KOCH-EBERTH's typhoid bacillus. It is, however, probable that, as in the case of tetanus, there are basic and other products formed. He obtained it by cultivating the bacillus on beef-broth for eight to fourteen days at the temperature 37.5–38°. The nature of the soil on which it grows has a great deal to do with the formation of the poison. An especially important factor is the temperature: for BRIEGER has observed that no poison was produced in one case where the temperature remained by accident at 39° for twenty-four hours. In such cases creatine is present in quantity, whereas otherwise the reverse is the rule.

In the process of extraction it occurs in the mercuric chloride precipitate, and from this it is obtained, after the removal of the mercury by hydrogen sulphide, as an easily deliquescent hydrochloride. This for the purpose of purification is converted into the difficultly soluble aurochloride.

Typhotoxine is isomeric with gadinine and the compound $C_7H_{17}NO_2$, which BRIEGER obtained from putrefying horseflesh. In its properties it is, however, very different. Thus, the free base is strongly alkaline and its hydrochloride yields a difficultly soluble picrate. On the other hand, the isomer from horseflesh possesses a slightly acid reaction, and does not form a picrate. Again, typhotoxine gives with EHRLICH's reagent (sulpho-diazobenzole) an immediate yellow color, which disappears upon the addition of alkali, whereas the isomer does not give this reaction. Furthermore, the two bases differ in their physiological action and in their behavior to alkaloidal reagents (see Table I.). Their aurochlorides, however, possess the same melting-point.

The HYDROCHLORIDE is readily deliquescent, and unites with platinum chloride to form an easily soluble double salt crystallizing in needles.

The AUROCHLORIDE, $C_7H_{17}NO_2.HCl.AuCl_3$ ($Au = 40.46$ per cent.), is difficultly soluble, and crystallizes in prisms, which melt at 176°. In its melting-point and solubility (197°, BRIEGER, *Arch. f. pathol. Anat.*, **115**, 489) it agrees with its isomer from horseflesh. From some of his first experiments in the cultivation of the typhoid bacillus, BRIEGER (II., 69) obtained a basic product differing in some of its characters from typhotoxine. Its aurochloride, on analysis, gave 41.91 and 41.97 per cent. of Au, 16.06 per cent. of C, and 3.66 per cent. of H.; while typhotoxine aurochloride gave 40.78 per cent. Au, 17.38 per cent. C, and 3.85 per cent. H. For a comparison of the reaction of these two substances see Table I.

In its physiological action, typhotoxine differs from its isomer (page 262) in that the latter produces symptoms with well-marked convulsions, whilst the former throws the animal into more of a paralytic or lethargic condition. The action of this base has been studied only on mice and guinea-pigs. It produces at first slight salivation with increased respiration; the animals lose control over the muscles of the trunk and extremities, and fall down helpless upon their sides. The pupils become strongly dilated, and cease to react to light; the salivation becomes more profuse; the rate of heart-beat and of respiration gradually decreases, and death follows in from one to two days. Throughout the course of these symptoms the animals have frequent diarrhœic evacuations, but at no time are convulsions present. On post-mortem, the heart is found to be in systole, the lungs are strongly hyperæmic, the other internal organs pale, the intestines firmly contracted, and their walls pale.

A BASE(?), $C_7H_{17}NO_2$, was obtained by BRIEGER in 1886 (III., 28) on working over about one hundred pounds of horseflesh which had been allowed to undergo slow putrefaction with limited access of air and at a low temperature (—9° to +5°) for four months. It occurs in the mercuric chloride precipitate together with cadaverine, putrescine, and mydatoxine, and from these bases it can be separated and isolated according to the method on page 233.

A similar, if not identical substance, having the composition $C_7H_{17}NO_2$, was obtained by BAGINSKY and STADTHAGEN (1890) from cultures on horseflesh, ten days at 35°, of a bacillus, closely allied to FINKLER-PRIOR'S, and isolated from stools of cholera infantum. The gold salt in crystalline form and properties is the same as BRIEGER'S, except that it possesses a somewhat higher melting-point.

The free substance possesses, even after most careful purification, a slightly acid reaction. This acidity is removed from even a large quantity of the substance by the addition of a drop of alkali. On account of the acid character of the free substance, BRIEGER does not consider it to be a base (a ptomaïne). It differs, however, from the amido-acids in its poisonous character; in the fact that, unlike an acid, it does not unite with bases to form salts; and in not giving the characteristic red coloration (HOFMEISTER'S reaction for the amido-acids) with ferric chloride. Whatever the true nature of this substance may be, it nevertheless, in its other properties, behaves like a base.

Thus, it forms simple as well as double salts. On boiling with copper acetate, it gives amorphous floccules. Under the desiccator it solidifies into plates which deliquesce on exposure to the air. It does not combine either with silver oxide or with cupric hydrate. On dry distillation it yields a distillate possessing a strong acid reaction and a peculiar odor. The distillate does not give any precipitate with platinum chloride, or with gold chloride, nor does it react with copper acetate. With phosphomolybdic acid, however, it forms an amorphous mass; with ferric chloride and potassium ferricyanide it yields an immediate precipitate of Berlin-blue, whereas the original substance does not give any blue coloration.

The HYDROCHLORIDE, $C_7H_{17}NO_2.HCl$, crystallizes in fine needles which are insoluble in absolute alcohol. When its aqueous solution is treated with freshly precipitated silver oxide, the resulting filtrate contains some silver oxide in solution, from which it can be removed by hydrogen sulphide; thus differing from an ammoniacal silver solution, which gives no precipitate on treatment with hydrogen sulphide. In this respect it resembles SALKOWSKI's base, page 231. For reactions of the hydrochloride, see Table I.

The AUROCHLORIDE, $C_7H_{17}NO_2.HCl.AuCl_3$, forms plates which are difficultly soluble in water, and melt at 176°—the melting point of the gold salt of typhotoxine. It is dimorphous, since sometimes it is also obtained in needles which can be changed into plates.

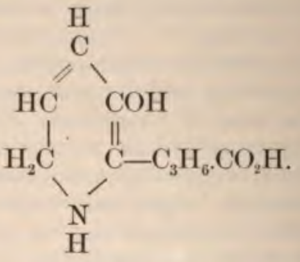
It does not form a picrate, nor does it give a reaction with sulpho-diazobenzole.

Physiological Action.—This substance, when injected into frogs, produces a curara-like action. A few minutes after the injection the animal falls into a condition of paralysis, and, although it can still react toward reflexes, it cannot move from its place. At times fibrillary twitchings pass over the body. The pupils dilate, the heart-action becomes gradually weaker, and finally, after several hours the animal dies, with the heart in diastole. Doses of 0.05 to 0.3 gramme of the hydrochloride, injected into guinea-pigs, produce in a short time a slight tremor, gradual increase in respiration, and slight moistening of the lower lip. The pupils at first contract, then dilate *ad maximum*, and become reactionless. The temperature remains at first normal; chills of short duration follow in rapid succession. The animal squats on the ground with its snout pressing against the floor in exactly similar manner as is caused by the mussel poison. Violent clonic convulsions follow in continually shorter intervals, and at the same time lachrymation and salivation become profuse, but not as excessive as in the case of the muscarine-like ptomaïnes. The temperature sinks with the decrease in the rate of respiration, the ears previously gorged become pale and cold, and the heart-action becomes irregular and less frequent than before. General paralysis sets in, but the head still moves upward and backward. External stimuli induce violent clonic convulsions, the animal repeats frequently choking movements, and at the same time yields large quantities of saliva; finally, it falls upon its side completely paralyzed, and dies. The heart stops in diastole, the intestines are pale and strongly contracted, and the bladder is empty and likewise contracted.

MORRHUIC ACID, $C_9H_{13}NO_3$, was obtained by GAUTIER and MOURGUES (1888) from brown cod-liver oil, together with six bases, already described—namely, butylamine, amylamine, hexylamine, dihydrolutidine, aselline and morrhuine. These bases constitute about 0.2 per cent. of the oil. The discoverers regard them as true leucomaïnes, dissolved from the hepatic cells by the oil. It is more probable, however, that these compounds are the products of initial decomposition, and for that reason they are described under the head of ptomaïnes. This compound is relatively abundant, and is basic as well as acid in character. It is resinous in appearance, and can be crystallized in flattened prisms, or large lance-shaped plates. When recently precipitated it is oleaginous, viscous, then gradually hardens. It possesses a disagreeable aromatic odor resembling that of the sea-weeds, upon which the fish feed. According to the discoverers its probable source is the lecithin derived thus from these weeds. It is soluble in alcohol, and but slightly in ether. It reddens turmeric, decomposes carbonates and with acids forms salts which precipitate lead acetate and silver nitrate, but not copper acetate, even on warming.

The hydrochloride is crystalline, and is partially dissociated by excess of water. The platinum salt is soluble, and crystallizes in very small cross-shaped prismatic needles. The gold salt is amorphous and is readily altered on heating.

The properties of this compound show that it is of a pyridine nature, and inasmuch as it does not give a precipitate with copper acetate, it would appear that the carboxyl is not directly united to the pyridine nucleus. This does not necessarily follow now that we know that some amido-acids exists which do not give a reaction with copper acetate (see page 231). Its pyridine nature is furthermore shown on distillation with lime. An oily alkaline base is thus obtained which forms an iodomethylate, and this with potassium hydrate yields quite an intense red color, resembling lees (De Coninck's reaction). On oxidation with permanganate of potassium it yields a monobasic acid. According to Gautier and Mourgues the compound is probably identical with De Jungh's gaduine, and they ascribed to it the following constitution, which, it should be said, lacks full confirmation:



Compare with tyrosin, $C_9H_{11}NO_3$ (page 197).

A **Base**, $C_5H_{12}N_2O_4$, was obtained by **Pouchet** (1884) from the residual liquors resulting from an industrial treatment of débris of bones, flesh, and waste of all kinds, with dilute sulphuric acid. It is accompanied by another base, $C_7H_{18}N_2O_6$, from which it can be separated by treatment with alcohol. The base itself forms tufts of delicate needles which alter or decompose less easily than the accompanying base. The platinochloride, $(C_5H_{12}N_2O_4.HCl)_2PtCl_4$, forms a dull yellow powder, somewhat soluble in strong alcohol, but insoluble in ether. The platinochloride $(C_7H_{18}N_2O_6.HCl)_2PtCl_4$ is insoluble in ether.

The hydrochlorides of these bases form silky needles which are altered by excess of hydrochloric acid and by exposure to air. **Pouchet** considers them to be closely allied to the oxy-betaines. The general alkaloidal reagents precipitate these bases; the phosphomolybdic precipitate, on addition of ammonia, gives a blue tint. Both bases are toxic, and exert a paralyzing action upon the reflex movements.

The method employed by **Pouchet** for their isolation was to precipitate them as tannates. The precipitate was decomposed by lead hydrate in the presence of strong alcohol, the excess of lead removed from the solution by hydrogen sulphide, and the clear liquid thus obtained was submitted to dialysis. The above bases occurred in the dialysate. In the non-dialyzable portion volatile bases were found probably identical with those described by **Gautier** and **Étard**.

Tetanine, $C_{13}H_{30}N_2O_4$, was obtained in 1886 by **Brieger** (III., 94) by cultivating impure tetanus microbes of **Rosenbach**, in an atmosphere of hydrogen on beef-broth for eight days at 37°–38°. It likewise occurs in cultures on brain-broth. Later (April, 1888), **Brieger** succeeded in obtaining tetanine from the amputated arm of a tetanus patient, identical in its physiological action and chemical reactions with that isolated from cultures of **Rosenbach**'s germs on beef-broth. The presence of tetanine during life in tetanus patients has thus been demonstrated. It has not been found in the brain and nerve tissue of persons dead from tetanus. A portion of the jelly-like mass taken from the amputated arm was found to contain tetanus bacilli as well as staphylococci and streptococci, and when planted on beef-broth, tetanine was formed, but no tetanotoxine or spasmotoxine.

KITASATO and WEYL (1890), employing pure cultures of the tetanus bacillus, obtained from 1¼ kilogramme beef used as culture medium 1.7118 gramme of tetanine hydrochloride (0.137 per cent.). Tetanotoxine was also present.

For its isolation BRIEGER employed the following method: The cultures were slightly acidulated with hydrochloric acid, heated and filtered; the filtrate was then treated with lead acetate and with alcoholic mercuric chloride in the manner described under mytilotoxine (page 255). KITASATO and WEYL digest the cultures with 0.25 per cent. hydrochloric acid for some hours at 60°, then render slightly alkaline, filter, and distil in vacuo at 60°. The residue in the retort is worked for tetanine by BRIEGER's method, while the distillate contains tetanotoxine, ammonia, indol, hydrogen sulphide, phenol and butyric acid. The filtrate from the above mercuric chloride precipitate contains the greater part of the active principle, provided the precipitate has been thoroughly washed. After the removal of the mercury by hydrogen sulphide, it is evaporated and the residue is repeatedly extracted with absolute alcohol, in which the tetanus poison readily dissolves, and can thus be separated from the insoluble ammonium chloride. The alcoholic solution is treated with alcoholic platinum chloride, which precipitates the ammonium and creatinine platinochlorides, whilst the platinochloride of the poison remains in solution. The filtrate from this precipitate gives, on the addition of ether, a flocculent precipitate possessing exceedingly deliquescent properties. The precipitate is, therefore, rapidly filtered off by means of a pump, and dried in vacuo. It can then be recrystallized from hot 96 per cent. alcohol, and the beautiful clear-yellow plates thus obtained, if dried again in vacuo, become rather difficultly soluble in water, from which it can then be recrystallized and obtained in a perfectly pure condition. If boiled with boneblack it decomposes, yielding a non-poisonous crystalline compound.

Phosphomolybdic acid cannot be used in the separation of tetanine, inasmuch as it destroys the poison (BRIEGER). BOCKLISCH has also observed that it destroys the poison formed in the putrefaction of fish.

Tetanine obtained by treating the hydrochloride with freshly precipitated moist silver oxide forms a strongly alkaline yellow syrup. With alkaloidal reagents it gives the same reactions as the hydrochloride, except that it does not give a blue color with ferric chloride and potassium ferricyanide. It is easily decomposed in acid solution, but is permanent in alkaline solution.

The HYDROCHLORIDE, $C_{13}H_{30}N_2O_4.2HCl$, is deliquescent and is easily soluble in absolute alcohol. Beside with platinum it combines only with phosphomolybdic acid to form an easily soluble crystalline precipitate, which on the addition of ammonium hydrate becomes white. If, however, the hydrochloride is impure, phosphomolybdic acid produces a precipitate which is colored an intense blue by ammonia. Potassium-bismuth iodide yields a precipitate which is at first amorphous, but soon becomes crystalline. Ferric chloride and potassium ferricyanide produce a slowly developing blue color which probably is due to impurities.

When kept for some months the highly poisonous hydrochloride becomes syrupy, brownish, and wholly inert. Examined at this stage, the syrup was found, by means of platinum chloride, to contain a substance the hydrochloride of which crystallized in plates. This is readily soluble in water and alcohol, and melts at 197°, with total decomposition, the same as tetanine. It combines only with phosphomolybdic acid to form an easily soluble compound. The platinum salt has the composition $C_6H_{13}NO_2.2HCl.PtCl_4$. This substance is non-poisonous and probably an amido-acid. It is different, however, from leucin and NENCKI's isomers of leucin, although possessing the same composition. It is also isomeric with mydatoxine, $C_6H_{13}NO_2$, but this is highly poisonous to mice, while the former is inert (see page 255). Tetanine resembles mytilotoxine with respect to this loss of toxicity on standing.

The PLATINOCHLORIDE, $C_{13}H_{30}N_2O_4.2HCl.PtCl_4$ (Pt = 28.33 per cent.), is easily soluble in absolute alcohol from which it is precipitated on the addition of ether. From ninety-six per cent. alcohol it crystallizes in clear yellow plates. After repeated recrystallization from alcohol and drying in vacuo it becomes difficultly soluble in water so that it can be recrystallized from the latter. It decomposes at 197°.

This base produces the characteristic, though probably not all the symptoms of tetanus, since we know of at least three other toxines (pages 194, 195) which occur with tetanine in cultures of the tetanus microbe. The symptoms induced by relatively large doses in warm-blooded animals, as mice, guinea-pigs, and rabbits, exhibit two distinct phases. In the first, the animal is thrown into a lethargic paralytic condition, then suddenly becomes uneasy, and the respiration becomes more frequent. This is followed by the second phase, in which tonic and clonic convulsions, especially the former, predominate till death results. 0.5 gramme has but slight action on guinea-pigs. Small doses do not seem to affect guinea-pigs, while frogs seem to be much less sensitive than mice. The characteristic convulsions and opisthotonus seen in tetanus in man are also produced in guinea-pigs on injection of large doses of this base. Dogs and horses seem to be but slightly sensitive to the action of this poison.

A BASE, $C_{14}N_{20}N_2O_4$, was isolated by GUARESCHI in 1887 from putrid fibrin. It occurs in the chloroform or ether extracts along with the base $C_{10}H_{13}N$, and is probably an amido-acid (see page 201).

A BASE, $C_7H_{18}N_2O_6$, was isolated by POUCHET in 1884. It is said to form short, thick prisms which become brown when exposed to light.

The PLATINOCHLORIDE, $(C_7H_{18}N_2O_6.HCl)_2PtCl_4$, crystallizes in prismatic needles which are insoluble in strong alcohol. For further details in regard to this base see page 265.

TYROTOXICON has been obtained in poisonous cheese (VAUGHAN, WALLACE, WOLFF), in poisonous ice-cream (VAUGHAN, NOVY, SCHEARER, LADD), in poisonous milk (VAUGHAN, NOVY, NEWTON, WALLACE, FIRTH, SCHEARER), and in cream-puffs (STANTON). The methods of separating this poison and its effect upon animals have already been given with sufficient detail. Chemically, it is very instable. When warmed with water to about 90°, it decomposes. Hydrogen sulphide also decomposes it, therefore all attempts to isolate it by precipitation with some base, such as mercury or lead, and then removing the base with hydrogen sulphide, have failed. Its unstable character is illustrated by the fact that it may disappear altogether within twenty-four hours from milk rich in the poison which is allowed to stand in an open beaker.

With potassium hydrate it forms a compound which agrees in crystalline form, chemical reactions, and the per cent. of potassium which it contains, with the compound of diazobenzole and potassium hydrate. This substance is best obtained from milk containing tyrotoxicon as follows: The filtered milk, which is acid in reaction, is neutralized with sodium carbonate, agitated with an equal volume of ether, allowed to stand in a stoppered glass cylinder for twenty-four hours, the ether removed, and allowed to evaporate spontaneously from an open dish. The aqueous residue is acidified with nitric acid, then treated with an equal volume of a saturated solution of potassium hydrate, and the whole concentrated on the water-bath (this compound is not decomposed below 130°). On being heated the mixture becomes yellowish-brown, and emits a peculiar aromatic odor. On cooling the tyrotoxicon compound forms in beautiful, six-sided plates along with the prisms of potassium nitrate.

With equal parts of sulphuric and carbolic acids, pure tyrotoxicon gives a green coloration, but in whey the color varies from yellow to orange-red. This color reaction may be used as a preliminary test in examining milk for tyrotoxicon. It is best carried out as follows: Place on a clean porcelain surface two or three drops each of pure carbolic and sulphuric acids. Then add a few drops of the aqueous solution of the residue left after the spontaneous evaporation of the ether. If tyrotoxicon be present, a yellow to orange-red coloration will be produced. This test is to be regarded only as a preliminary one, for the coloration may be due to the presence of a nitrate or nitrite, or as HUSTON has shown, to butyric acid. The tyrotoxicon must be converted into the potassium compound and purified before the absence of nitrate or nitrite can be positively demonstrated. Moreover, the physiological test should always be made in testing for this poison.

With platinum chloride in alcoholic solution tyrotoxicon forms a compound which explodes with great violence at the temperature of the water-bath. This also corresponds with the compound of platinum chloride and diazobenzole.

Pure tyrotoxicon is insoluble in ether, and its extraction from alkaline solutions by this solvent is due to the presence of foreign matter, with which the poison is taken up by the ether.

The physiological action of this ptomaïne has been sufficiently discussed in a preceding chapter.

MYDALEINE (μυδαλέος, putrid) is a poisonous base obtained in 1885 from putrefying cadaveric organs, liver, spleen, etc. (BRIEGER, II., 31, 48). Though it is apparently present on about the seventh day, it is unobtainable until about the third or fourth week. The method for its separation from the accompanying bases is given under Saprine (page 220). It is liable to occur in the mercuric chloride filtrate, as well as in the precipitate, inasmuch as the double salt is insoluble only in perfectly absolute alcohol. In order to purify the platinochloride obtained as on page 221, it is repeatedly recrystallized from a very small quantity of lukewarm water. This base has not been obtained in sufficient quantity to permit of a complete determination of its composition. It is probably a diamine, containing four or five carbon atoms, and hence is nearly related to some of the diamines already described.

The PLATINOCHLORIDE, on analysis, gave: Pt = 38.74 C = 10.83, H = 3.23. It crystallizes in small needles, and is extremely soluble in water.

The HYDROCHLORIDE crystallizes with extreme difficulty, even on standing for some time in a desiccator. On exposure to the air it rapidly deliquesces.

Physiological Action.—Mydaleine has an entirely specific action. Small quantities injected into guinea-pigs or rabbits produce, after a short time, a moistening of the under lip, and an abundant flow of secretion from the nose and eyes. The pupils dilate gradually to maximum, and become reactionless; the ear vessels become strongly injected, and the body temperature rises 1° to 2°. The hairs bristle, and the animal occasionally shudders. Gradually the salivation ceases, the respiration and heart-action, which were at first hastened, now decrease, the temperature falls, the ears become pale, and the animal finally recovers. During the action of the poison the animal shows a tendency to sleep, and the peristaltic action of the intestines is heightened. Larger doses (0.050 gramme) induce an exceedingly violent action, which invariably results in the death of the animal. On post-mortem, the heart is found to be stopped in diastole, and the intestines and bladder contracted; otherwise nothing abnormal is observed.

A TOXIC BASE.—From human livers and spleens which were decomposing for two weeks in thorough contact with air there was isolated, besides cadaverine and putrescine, a small quantity of a poisonous base (BRIEGER, II., 29, 48). The mercuric chloride precipitate was decomposed, and the hydrochlorides were precipitated by gold chloride (to remove cadaverine, which is soluble), and the aurochloride was then changed into the platinum salt, whereby the insoluble putrescine platinochloride was removed. In the mother-liquors from the putrescine salt an easily soluble platinum compound was separated, and found to contain 41.30 per cent. Pt. It crystallized in fine needles. The hydrochloride formed small, readily deliquescent needles, and did not produce a precipitate in alcoholic platinum chloride. Injected into guinea-pigs and rabbits it induced an exalted peristaltic action of the intestines, which lasted several days, and produced in the animals, on account of the continuous evacuations, a condition of great weakness. No disturbance in the functions of the other organs was observed.

A Base was isolated from decomposing haddock which were exposed for five days during summer in an open iron vessel. Brieger (I., 42) found in the aqueous mercuric chloride precipitate (see page 258) a base the hydrochloride of which crystallized in well-formed, small needles. The platinochloride likewise crystallized in beautiful needles, and gave, on analysis, 36.03 per cent. of Pt; 7.81 per cent. of N.

A substance of muscarine-like action was obtained by Brieger (I., 59) from putrefying gelatin, ten days at 35°, though in insufficient quantity to permit a determination of its character. The residue containing this substance gave, on distillation with alkali, only ammonia.

A Base was obtained by Bocklisch (III., 52, 53) from herring which had undergone putrefaction for twelve days. It was found in the distillate, together with trimethylamine and dimethylamine, obtained by distilling the mercuric chloride filtrate, after the removal of the mercury, with sodium hydrate. The platinochloride was easily soluble, and crystallized in large thin plates. On analysis it gave: $Pt = 28.57$, $C = 22.34$, $H = 4.66$. The hydrochloride is easily soluble in water, and in absolute alcohol, and besides with platinum gives only with phosphomolybdic acid a yellow precipitate which is soluble in excess, and with ammonia gives an immediate blue color. It immediately reduces a mixture of ferric chloride and potassium ferricyanide with formation of Berlin blue; and similarly throws down metallic gold from solutions of gold chloride.

From poisonous mussel, BRIEGER (III., 79) obtained an aurochloride of a base crystallizing in needles. The quantity isolated was insufficient for analysis. It is interesting because of its property of inducing salivation, a symptom which has been observed by SCHMIDTMANN and by CRUMPE in some cases of mussel poisoning.

A BASE was obtained by GUARESCHI and MOSSO (*Journ. für praktische Chem.*, **28**, 508) from fresh beef, in the alkaline ether extract obtained by DRAGENDORFF'S method. It formed a yellowish alkaline fluid, of unpleasant odor, and after a time gave a deposit of microscopic crystals. The hydrochloride gave the following reactions: Gold chloride, yellow crystalline precipitate; platinum chloride, precipitate; potassium iodide and iodine in hydriodic acid, kermes-red precipitate; phosphotungstic acid, nothing; phosphomolybdic acid, an abundant yellow precipitate; tannic acid, heavy, grayish precipitate, same with MAYER'S reagent; picric acid, yellow precipitate; MARMÉ'S reagent, precipitate soluble in excess; potassium bichromate, nothing; potassium permanganate and sulphuric acid, violet color; potassium ferricyanide and ferric chloride, Prussian blue precipitate.

By giving a precipitate with tannin, and not with phosphotungstic acid, it resembles neurine.

CH. GRAM has studied the decomposition of yeast under the influence of an infusion of hay. The yeast was allowed to putrefy for fourteen days, and was then treated with zinc sulphate. The latter was precipitated by barium hydrate, and the filtrate after the removal of the barium by sulphuric acid, was evaporated to dryness, and extracted with absolute alcohol. The alcoholic solution was evaporated, and the residue again extracted with alcohol. The extraction residue was taken up with water, and again subjected to the above treatment with zinc sulphate, barium hydrate, etc.

The filtrate was poisonous, and produced, in frogs, paralysis and stoppage of the heart in diastole Addition of platinum chloride and alcohol precipitated two bases. One of these, although possessing a curara-like action, did not affect the heart. When its solution was heated for twenty-four hours on the water-bath, it caused general paralysis and stoppage of the heart. The platinochloride contained 38.05 per cent. of platinum.

The other base also possessed a slight curara-like action, and its platinochloride gave, on analysis, 40.92 and 39.4 per cent. of platinum.

BRIEGER found a basic substance in small quantities in cultures of the staphylococcus pyogenes aureus on bouillon and beef-broth (II., 74). The hydrochloride formed groups of colorless, non-deliquescent needles. With platinum chloride it yielded a double salt, crystallizing in needles, and containing 32.93 per cent. of Pt. For its reactions, see Table I.

From aqueous as well as alcoholic solutions of cultures of staphylococcus aureus LEBER (1888) isolated a crystalline substance which he named *phlogosine*. The composition of this substance is not known. It does not seem to contain nitrogen, and inasmuch as it blackens silver it probably contains sulphur. It crystallizes in fine needles which are soluble in ether and in alcohol; difficultly soluble in water. It sublimes in needles. Alkalies precipitate it as amorphous yellow floccules which are soluble in acid and then can be recrystallized. With potassium ferricyanide and ferric chloride it yields a blue color, and with potassio-mercuric, cadmic, and bismuth iodides precipitates which are soluble in excess. No precipitate is produced by gold or platinum chlorides, phosphotungstic or molybdic, tannic or picric acids.

A small quantity applied to the conjunctiva produces intense inflammation, suppuration, and necrosis. Introduced into the anterior chamber it induces intense suppuration and keratitis. The substance is entirely distinct from the base obtained by BRIEGER, described above.

A Base—boiling point about 284°—was obtained by Brieger (II., 61) from human livers and spleens which were putrefying for two to three weeks. It occurs in the mercuric chloride filtrate, as described under Saprine, page 220, together with some mydaleine, trimethylamine, and hydrocarbons. The filtrate, after the mercury is removed by hydrogen sulphide, is evaporated to dryness, and finally the last traces of water are removed in a vacuum. The residue is then treated with absolute alcohol, and from this alcoholic solution the mydaleine is precipitated by the addition of alcoholic mercuric chloride. The trimethylamine is separated by distillation of the alkaline filtrate, previously deprived of its mercury by hydrogen sulphide; while the mother-liquor yields an oily mixture of hydrocarbons and bases. The latter were separated by fractional distillation, whereby only one of the bases was obtained in sufficient quantity for study. It boiled at about 284°, and gave with hydrochloric acid, on evaporation, a salt crystallizing in beautiful, long needles, which were very easily soluble in perfectly absolute alcohol. With gold chloride and picric acid it gave only oily products; with ferric chloride and potassium ferricyanide, an intense blue; with platinum chloride, an extremely easily soluble double salt, which appeared under the microscope in the form of very fine needles, while from alcohol-ether the double salt slowly separated in thin plates which contained 30.36 per cent. of platinum. The free base showed a slight fluorescence. It is not poisonous, and, according to Brieger, is probably a pyridine derivative.

Other non-poisonous bases were present in very small quantity in the mother-liquor described above, after the separation of the oily mixture.

Peptotoxine.—By this name Brieger (I., 14–19) has designated a poisonous base which he has found in some peptones, and hence in the digestion of fibrin; in putrefying albuminous substances, such as fibrin, casein, brain, liver, and muscles. It is a well-known fact that animal tissues, in the early stages of putrefaction, possess strong toxic properties, even before the decomposition could have advanced far enough to effect a splitting-up of the proteid and carbohydrate molecules. BRIEGER and others have tried to seek an explanation of this toxicity by connecting it with an early peptonization of the proteids brought about by the action of ferments which are distributed throughout the tissues, and which begin their activity immediately after death. This poison has not been definitely isolated, but its general properties and action have been studied by BRIEGER and SALKOWSKI. The former prepared it by digesting fibrin for twenty-four hours with gastric juice at the temperature of the blood. The perfectly fresh peptone thus obtained was evaporated to a syrupy residue, and this was then extracted with boiling alcohol. The residue left on evaporation of the alcoholic solution was digested for some time with amyl alcohol, which on subsequent evaporation gave amorphous brownish masses. This extract can then be purified by neutral lead acetate. The filtrate, after the removal of the lead by hydrogen sulphide, is repeatedly extracted with ether, then evaporated to dryness, and extracted as before, with amyl alcohol. This final extract is evaporated to drive off the alcohol, taken up with water, and filtered. The colorless aqueous solution thus obtained contains the poisonous substance, which, however, can only with extreme difficulty be brought to crystallization in vacuo.

This poison, when in its purest condition, as shown by its failure to give the biuret reaction, possesses a neutral reaction. Its behavior to Millon's reagent is quite characteristic: it gives a white precipitate, which on boiling becomes intensely red. From this reaction, BRIEGER is inclined to regard this substance as a hydroxyl or an amido-derivative of benzole. The ptomaïne can be extracted from acid as well as alkaline solution by amyl alcohol—more difficult in the cold than on heating. It is absolutely insoluble in ether, benzol, and chloroform; very soluble in water. It is not destroyed by boiling, by passing hydrogen sulphide, or by strong alkalies; but is destroyed, however, when the putrefaction lasts longer than eight days. For its behavior to reagents, see Table I.

Various observers have shown that peptone possesses a toxic action, and some have been led to regard this toxicity as not due to the peptone itself, but rather to the presence of this or some other ptomaïne. At least BRIEGER found one specimen of dry Witte's peptone to be perfectly harmless; whereas, the fresh peptone formed by fibrin digestion possessed strong toxic powers. Moreover, this non-poisonous peptone when exposed to the action of gastric juice was found to yield the poisonous substance. The poisonous nature of proteids and the physiological action of this base will be described later.

PYOCYANINE, $C_{14}H_{14}NO_2$, is the coloring matter of blue pus, and is produced by the action of bacillus pyocyaneus. It was isolated by LEDDERHOSE (1887) and is said to be an anthracene derivative. On contact with the air it is oxidized to pyoxanthose, a yellow substance. According to KUNZ it contains nitrogen and sulphur. The picrate is of a dark reddish-brown color; the platinum salt is black and sometimes is obtained as glittering fine golden needles.

TABLE OF PTOMAÏNES.

Formula.	Name.	Discoverer.	Physiological action.[1]
$C\ H_5\ N$	Methylamine.	Bocklisch.	Nou-poisonous.
$C_2\ H_7\ N$	Dimethylamine.	Brieger.	“ “
$C_3\ H_9\ N$	Trimethylamine.	Dessaignes.	“ “
$C_2\ H_5\ N$	Spermine(?).	Kunz.	“ “
$C_2\ H_7\ N$	Ethylamine.	Hesse.	“ “
$C_4\ H_{11}N$	Diethylamine.	Bocklisch.	“ “
$C_6\ H_{15}N$	Triethylamine.	Brieger.	“ “
$C_3\ H_9\ N$	Propylamine.	“	
$C_4\ H_{11}N$	Butylamine.	Gautier & Mourgues.	Poisonous(?).
$C_5\ H_{11}N$ (?)	Tetanotoxine.	Brieger.	Poisonous.
$C_5\ H_{13}N$	Amylamine.	Hesse.	“
$C_6\ H_{15}N$	Hexylamine.	“	“
$C_7\ H_{11}N$	Di-hydrolutidine.	Gautier & Mourgues.	“
$C_8\ H_{11}N$	Collidine(?).	Nencki.	
$C_8\ H_{11}N$	Pyridine base(?).	O. de Coninck.	
$C_8\ H_{13}N$	Hydrocollidine(?).	Gautier and Etard.	Poisonous.
$C_9\ H_{13}N$	Parvoline(?).	“ “ “	
$C_{10}H_{15}N$	Unnamed.	Guareschi and Mosso.	Poisonous.
$C_{10}H_{15}N$	Pyridine base(?).	O. de Coninck.	
$C_{10}H_{17}N$	Hydrocoridine(?).	Griffiths.	
$C_{32}H_{31}N$	Unnamed.	Delézinier.	
$C_2\ H_8\ N_2$	Ethylidenediamine(?).	Brieger.	Poisonous.
$C_3\ H_8\ N_2$	Trimethylenediamine(?).	“	“
$C_4\ H_{12}N_2$	Putrescine.	“	Not very poisonous.
$C_5\ H_{14}N_2$	Cadaverine.	“	“ “
$C_5\ H_{14}N_2$	Neuridine	“	Non-poisonous.
$C_5\ H_{14}N_2$	Saprine.	“	“ “
$C_7\ H_{10}N_2$	Unnamed.	Morin.	“ “
$C_{10}H_{26}N_2$(?)	Susotoxine.	Novy.	Poisonous.
$C_2\ H_7\ N_3$	Methyl-guanidine.	Brieger.	“
$C_{19}H_{27}N_3$	Morrhuine.	Gautier & Mourgues.	Diuretic, etc.
$C_{13}H_{20}N_4$	Unnamed.	Oser.	
$C_{17}H_{38}N_4$	“	Gautier and Etard.	
$C_{25}H_{32}N_4$	Aselline.	Gautier & Mourgues.	Poisonous.
$C_5\ H_{13}N\ O$	Neurine.	Brieger.	“
$C_8\ H_{11}N\ O$	Mydine.	“	Non-poisonous.
$C_5\ H_{11}N\ O_2$	δ-amido-valerianic acid.	E. and H. Salkowski.	“ “
$C_5\ H_{15}N\ O_2$	Choline.	Brieger.	Poisonous.
$C_6\ H_{13}N\ O_2$	Mydatoxine.	“	“
$C_6\ H_{13}N\ O_2$	Unnamed.	Brieger, 1888. (tetanus cult.)	Non-poisonous.
$C_6\ H_{15}N\ O_2$	Mytilotoxine.	Brieger.	Poisonous.

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[1] Only those bases are here denoted as poisonous which possess a decided toxicity.

TABLE OF PTOMAÏNES—*Continued.*

Formula.	Name.	Discoverer.	Physiological action.[1]
$C_7H_{17}NO_2$	Gadinine.	Brieger.	Poisonous.
$C_7H_{17}NO_2$	Typhotoxine.	"	"
$C_7H_{17}NO_2$	Unnamed.	"	"
$C_{14}H_{14}NO_2$	Pyocyanine.	Ledderhose.	Non-poisonous.
$C_5H_{13}NO_3$	Betaine.	Brieger.	"
$C_5H_{15}NO_3$	Muscarine.	"	Poisonous.
$C_9H_{13}NO_3$	Morrhuic acid.	Gautier & Mourgues.	
$C_5H_{12}N_2O_4$	Unnamed.	Pouchet.	Poisonous.
$C_{13}H_{30}N_2O_4$	Tetanine.	Brieger.	"
$C_{14}H_{20}N_2O_4$	Unnamed.	Guareschi.	
$C_7H_{18}N_2O_6$	"	Pouchet.	Poisonous.
	"	"	"
	Tyrotoxicon.	Vaughan.	"
	Mydaleine.	Brieger.	"
	Spasmotoxine.	"	"
	A diamine(?).	" (tetanus cult.)	"
	Peptotoxine.	"	"
	Phlogosine.	Leber.	Inflammatory.

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[1] Only those bases are here denoted as poisonous which possess a decided toxicity.

CHAPTER XII.

CHEMISTRY OF THE LEUCOMAÏNES.

UNDER this head are classed those basic substances which are found in the living tissues, either as the products of fermentative changes or of retrograde metamorphosis. Most of these substances have already been known for many years, though their real significance as alkaloidal bodies, and their relation to the functional activities of the animal organism have been but little understood, or rather they have not been brought together under the leading conception that they are alkaloidal products of physiological change. The first attempt at the systematic study and generalization of these basic substances was made by GAUTIER, who applied to them the name leucomaïnes, a term derived from the Greek λεύχωμα, signifying white of eggs. Under this name he includes all those basic substances which are formed in animal tissues during normal life, in contradistinction to the ptomaïnes or basic products of putrefaction. The distinction between vegetable and animal alkaloids is not very well defined, and, in fact, there seem to be reasons for considering their formation as due to the same causes which bear an intimate relation to the physiology of the cells and tissues of both kingdoms. Thus, vegetable tissues are known to contain not only definite ptomaïnes, such as choline, but also leucomaïnes, as hypoxanthine, xanthine, etc. Indeed, in this latter group must be placed, on account of their relation to xanthine, those well-defined alkaloidal bases, caffeine and theobromine. Not only are the representatives of these two divisions of basic substances common to both kingdoms, but their parent bodies, lecithin, nuclein, etc., are known to occur in both, thus giving rise to the same bases on decomposition.

So far as the genesis of most of the leucomaïnes is concerned, we know very little, though **GAUTIER** is of the belief that they are being formed continuously and incessantly in the animal tissues side by side with the formation of urea and carbonic acid and at the expense of the nitrogenous elements. It is quite probable, as **KOSSEL** has pointed out, that some of these products are in themselves antecedents of end-products of metabolism. This is unquestionably true of the imido group, which exists in the adenine and guanine molecules, and through vital or putrefactive processes is split off, giving rise to ammonia, which in turn serves to form urea and uric acid. **BOUCHARD** has sought an explanation of the presence of these bases in the urine, by supposing that they were originally formed in the intestinal tract, from which they were absorbed into the system, to be subsequently eliminated by the kidneys. This view has also been brought forward by **SCHÄR** (1886), who holds that these bases, which may be formed by putrefactive changes in the intestinal tract, are absorbed into the circulatory system, whence they may be partly eliminated by the kidneys or may be partly deposited in the tissues themselves.

The views of **BOUCHARD** and **SCHÄR** have, to a certain extent, been confirmed by the investigations of **UDRANSZKY** and **BAUMANN**, who showed that the well-known ptomaïnes, cadaverine and putrescine, occur in the urine in cystinuria, and are formed by putrefactive changes induced in the intestinal tract probably by specific microörganisms. Under this same head fall the recent observations of **WOLKOW** and **BAUMANN**, that alkapton is produced from tyrosin by similar changes in the intestines. The origin of the true leucomaïnes cannot, however, be accounted for in this manner, for they are indissolubly connected with the metabolism of the cell itself, and are, therefore, formed in the tissues and organs proper, especially those rich in nucleated cells.

Another source of the nitrogenous bases must not be lost sight of, and that is protoplasm itself. The researches of **DRECHSEL**, **SIEGFRIED**, and **SCHULZE** have shown that nitrogenous bases do result from the decomposition of animal and vegetable proteids (see p. 242).

The leucomaïnes proper can be divided into two distinct and well-defined groups: (1) the Uric Acid Group, and (2) the Creatinine Group.

The first of these divisions contains a number of well-known bases which are closely related to uric acid. The order in which they will be described is as follows:

Adenine,	$C_5H_5N_5$.
Hypoxanthine,	$C_5H_4N_4O$.
Guanine,	$C_5H_5N_5O$.
Xanthine,	$C_5H_4N_4O_2$.
(Uric Acid,	$C_5H_4N_4O_3$.)
Heteroxanthine,	$C_6H_6N_4O_2$.
Paraxanthine,	$C_7H_8N_4O_2$.
Carnine,	$C_7H_8N_4O_3$.
Pseudoxanthine,	$C_4H_5N_4O$.
Gerontine,	$C_5H_{14}N_2$.
Spermine,	C_2H_5N (?).

The members of the second group have all been discovered by GAUTIER, and by him are regarded as allied to creatine and creatinine. These two substances, especially the latter, have been hitherto regarded as strongly basic in character, but SALKOWSKI has recently shown that creatinine, when perfectly pure, possesses little or no alkaline reaction, and, moreover, does not combine with acids. The bases in this group are:

(Creatinine,	$C_4H_7N_3O$.)
(Creatine,	$C_4H_9N_3O_2$.)
Cruso-creatinine,	$C_5H_8N_4O$.
Xantho-creatinine,	$C_5H_{10}N_4O$.
Amphi-creatine,	$C_9H_{19}N_7O_4$.
Base,	$C_{11}H_{24}N_{10}O_5$.
Base,	$C_{12}H_{25}N_{11}O_5$.

Besides these two general classes of leucomaïnes, there may be made a third class of undetermined leucomaïnes, embracing those bases which have been observed, but studied more or less incompletely, in the various physiological secretions of the body.

LEUCOMAÏNES OF THE URIC ACID GROUP.

ADENINE, $C_5H_5N_5$, which was discovered by **KOSSEL** in 1885, forms the simplest member of the uric acid group of leucomaïnes, and as such it deserves special attention, inasmuch as it shows most clearly the relation that exists between hydrocyanic acid and the members of this group. This base is apparently formed by the polymerization of hydrocyanic acid—a view that is confirmed, at least in part, by the fact that on heating with potassium hydrate to 200°, it yields a large quantity of potassium cyanide. Moreover, by the action of reducing agents, it is converted into a substance similar to, if not identical with, azulmic acid. It has not been prepared synthetically, though **GAUTIER** has claimed to have synthesized two closely related bodies, xanthine and methyl-xanthine, by simple heating of hydrocyanic acid in a sealed tube in contact with water and a little acetic acid.

This base was first prepared from pancreatic glands—hence the term adenine, which is derived from the Greek word *αδήν*, meaning a gland. It has since been shown to occur together with guanine, hypoxanthine, etc., as a decomposition-product of nuclein, and, therefore, it may be obtained from all tissues and organs, animal or vegetable, rich in nucleated cells. Accordingly, it has been found in the kidneys, spleen, pancreatic, thymus and lymphatic glands, in beer-yeast, in spermatic fluids, but not in testicles of the steer; occurs also in tea-leaves. In the latter adenine appears to exist in a preformed condition, since it can be extracted without the use of acid reagents. The thymus gland, as a prototype of embryonic, highly cellular tissue, yields a considerable amount of adenine; that from a calf, for instance, was found by **SCHINDLER** to contain 0.18 per cent. It has also been observed in the liver and urine of leucocythæmic patients; its occurrence in this disease will be readily understood when it is remembered that leucocythæmia is characterized by the presence in the blood of an unusual proportion of the nucleated white blood-corpuscles, which, owing to various unfavorable conditions, become destroyed in time, and the contained nuclein, as a result, splits up into adenine and guanine. These two bases may, therefore, be expected in all pathological conditions where there is an abnormal accumulation of pus. Indeed, as early as 1865, NAUNYN extracted from pus, obtained from the pleural cavity, a considerable quantity of a substance which was probably either adenine or guanine, or both. Adenine does not occur, or only in minute traces, in meat extract; and in this it resembles guanine, which is present only in traces. This may be due to the fact that adenine and guanine are readily converted into hypoxanthine and xanthine respectively, as has been shown in the putrefaction experiments of SCHINDLER. They may be considered as transitional products of cell-metabolism, the imido group contained in each readily being replaced by oxygen, and giving rise to ammonia, and this in turn to urea. KOSSEL, however, explains this fact on the ground that the muscle tissue is very poor in nucleated cells, *i. e.*, in nuclein. It would seem that the muscle cell in losing the morphological character of a cell has also suffered a corresponding loss in its chemical properties. For while the decomposition-products of nuclein—hypoxanthine, xanthine, phosphoric acid, etc.—are found in the muscle tissue, they do not exist in combination as they do in the nuclein molecules. This is seen in the fact that the bases exist in the free condition, since they can be extracted by water; and again, the phosphoric acid is present in the muscle tissue, not in organic combination, but as a salt. In the nucleated cell, adenine, guanine, etc., do not exist in the free condition, but form, in part at least, with albumin and phosphoric acid, a loose combination which is readily decomposed by the action of acids at the boiling temperature. This same change takes place spontaneously after death.

There can be no doubt that adenine and guanine play an important part in the physiological function of the cell nucleus, which, from recent observations, appears to be necessary to the formation and building up of organic matter. It is now known that non-nucleated cells, though capable of living, are not capable of reproduction. We must look, therefore, to the nucleus as the seat of the functional activity of the cell—indeed, of the entire organism. Nuclein, the parent substance of adenine and guanine, is the best known and probably most important constituent of the nucleus, and as such it has been already credited with a direct relation to the reproductive powers of the cell. This chemical view has recently been confirmed by ZACHARIAS, who showed that chromatin of histologists is identical with nuclein. LIEBERMANN has questioned nuclein as being the source of xanthine compounds, but in this he is not supported by the mass of evidence.

The method employed by KOSSEL for the preparation of adenine, is as follows: The finely divided pancreatic glands are heated to boiling, for three or four hours, with a large quantity of dilute sulphuric acid (0.5 per cent. by volume of concentrated acid), and the acid solution thus obtained is treated with a slight excess of hot concentrated baryta water. The excess of baryta is removed by carbonic acid, and the solution is then filtered; the filtrate is concentrated to a small bulk, about 100 c.c., rendered alkaline with ammonium hydrate, and finally precipitated with an ammoniacal solution of silver nitrate. The precipitate, consisting of the silver compound of the xanthine bodies, is partially dried by spreading over porous porcelain plates; then dissolved in warm nitric acid of specific gravity 1.1, to which a little urea has been added to prevent the formation of hypoxanthine should traces of nitrous acid be present. The filtered acid solution, treated with silver nitrate, on cooling, gives a deposit of the silver salts of hypoxanthine, guanine, and adenine, which is filtered off and thoroughly washed. The adenine separates out almost quantitatively if a little silver nitrate solution is added. The filtrate contains any xanthine silver compound that may be present. The washed precipitate of the silver salts is suspended in water, nitric acid added, decomposed with hydrogen sulphide (ammonium sulphide, or, better, hydrochloric acid, may be used), and the clear filtrate is concentrated on the water-bath to a small volume. It is then saturated with ammonium hydrate and digested on the water-bath for some time, whereby adenine and hypoxanthine go into solution, while the guanine remains undissolved (see p. 287). From the ammoniacal solution on partial concentration and subsequent cooling, the adenine crystallizes out first, whereas the more soluble hypoxanthine remains in solution. If the adenine is still colored it can be purified by dissolving in water and boiling with animal charcoal. The hot aqueous solution is then rendered very slightly alkaline with ammonium hydrate and allowed to cool; adenine crystallizes out, and can be still further purified by recrystallization from water.

Ammonium sulphide has been employed by **Schindler**, in place of hydrogen sulphide, in decomposing the silver compounds of the above bases. **Bruhns** recommends instead warming with very dilute hydrochloric acid, especially if guanine is present. The solution can then be neutralized with $NaHCO_3$, using methyl-orange as indicator, and the adenine separated from hypoxanthine by the picric acid method described below.

Another method for the separation of adenine from hypoxanthine is based upon the behavior of the nitrates of these bases in aqueous solution. From concentrated aqueous solutions of the nitrates, free hypoxanthine crystallizes out first, because the nitrate is decomposed; whereas, adenine, which is a stronger base, remains in combination with the acid, in solution.

Schindler determines adenine and hypoxanthine indirectly. The ammoniacal solution which is filtered from the insoluble guanine is evaporated to dryness on a weighed platinum dish, dried at 110°, and weighed. A nitrogen determination is now made of the mixed bases and from these data the proportion of each is calculated.

By far the best method for the quantitative separation of adenine and hypoxanthine is the picrate method of **Bruhns**.

The solution of the salts of the bases, preferably as nitrates or sulphates, must be neutral or faintly acid; excess of alkali or acid interferes. Such a solution can be obtained by evaporating the filtrate from the guanine in Kossel's method (page 286), and dissolving the residue in nitric acid; this is neutralized with sodium carbonate, using methyl-orange as indicator. On the addition of excess of sodium picrate the adenine is thrown down as a clear yellow flocculent precipitate. If the precipitation is made at the boiling temperature, on cooling the adenine salt separates in a crystalline condition and is more easily filtered and washed. After standing fifteen minutes the precipitate is filtered off by the aid of a suction-pump on a weighed filter, washed with cold water, and dried at 100°. As a correction for the solubility of the adenine picrate, 2.4 mg. per 100 c.c. filtrate can be added to the calculated amount of adenine.

The hypoxanthine picrate is very soluble, and, therefore, remains in solution. In this it is estimated according to the method described on page 302.

Adenine, when crystallized from warm or impure solutions, is obtained either as an amorphous substance, pearly plates, or in the form of very small microscopic needles; from dilute cold solutions it separates in long, needle-shaped crystals containing three molecules of water. This water of crystallization is lost on exposure to the air or on heating to 53°, and the crystals become opaque. It is soluble in about 1086 parts of water at the ordinary temperature; more easily in hot water, from which, on cooling, it recrystallizes. The aqueous solution possesses a neutral reaction. The free base is insoluble in ether, chloroform, and alcohol; soluble in glacial acetic acid, and somewhat in hot alcohol. It dissolves readily in mineral acids, yielding well-crystallizable salts. The fixed alkalies dissolve it with ease, but on neutralization of the solution it is reprecipitated In aqueous ammonium hydrate it is more readily soluble than guanine (which is insoluble, Schindler), and more difficultly soluble than hypoxanthine—a fact which is made use of to effect a separation from these bases. It is but slightly soluble in sodium carbonate.

Adenine can be heated to 278° without melting; at this temperature it becomes slightly yellow, and yields a white sublimate. It can be completely volatilized without decomposition, by heating on an oil-bath to 220°; the sublimate consists of pure, white, plumose needles of adenine, but at 250° partial decomposition occurs, and some hydrocyanic acid forms. When heated with potassium hydrate to 200° on an oil-bath, it yields a considerable quantity of potassium cyanide. Adenine is quite indifferent to the action of acids, alkalies, and even oxidizing agents. Thus, it may be boiled for hours with baryta, potash, or hydrochloric acid, without suffering decomposition. But when heated with dilute hydrochloric acid, or concentrated hydriodic acid, in a sealed tube at a temperature exceeding 100°, adenine is completely decomposed, with formation of carbonic acid and ammonia:

$$C_5H_5N_5 + 5H_2O + 5O = 5CO_2 + 5NH_3.$$

The free base, as well as benzoyl-adenine, is unaffected by the weak oxidizing action of potassium permanganate, but on stronger oxidation it is wholly destroyed. Bromine water produces in aqueous solutions of adenine an oily precipitate, which, on contact with potassium hydrate or ammonia, gives a beautiful red or violet color. Sodium amalgam and zinc chloride appear to have no action; but on boiling with zinc and hydrochloric acid it yields a very unstable reduction-product, which in the presence of oxygen first assumes a red color, and finally throws down a reddish-brown precipitate. This brown substance appears to be identical with azulmic acid, which has been known for a long time as a product of the polymerization of hydrocyanic acid.

When adenine is evaporated on the water-bath with dilute or fuming nitric acid, it gives a white residue which fails to give any coloration with sodium, ammonium, or barium hydrate. Similarly, it does not give the so-called WEIDEL's reaction (murexide test) on evaporation with chlorine water and exposure of the residue to an ammoniacal atmosphere.

In this respect it resembles hypoxanthine, which, when pure, does not answer to either of these tests. Another test for adenine, which, however, is given also by hypoxanthine but not by guanine and caffeine, is as follows: The substance to be tested is digested for half an hour with zinc and hydrochloric acid in a test-tube on the water-bath. If adenine is present, the solution will assume on standing, more rapidly on shaking, a ruby-red coloration, which later on turns into a brownish-red. This reaction depends upon the formation of a reduction-product, which, owing to its unstable nature, is soon oxidized by the oxygen of the atmosphere into a brownish, amorphous substance, apparently identical with azulmic acid.

On treatment with nitrous acid, it is converted into hypoxanthine according to the equation:

$$C_5H_5N_5 + HNO_2 = C_5H_4N_4O + N_2 + H_2O.$$

This formation of hypoxanthine from adenine is analogous to Strecker's transformation of guanine into xanthine by a similar action of nitrous acid (see Guanine). In both cases the change of a highly nitrogenized into a less nitrogenized body is accomplished by replacing an NH group by O, or, more exactly, of an NH_2 group by OH. In fact, the change is identical with that seen in the conversion of primary amines into primary alcohols. Thus,

$$\underset{\text{ETHYLAMINE.}}{C_2H_5.NH_2} + HNO_2 = \underset{\text{ETHYL ALCOHOL.}}{C_2H_5OH} + N_2 + H_2O.$$

In the extraction of adenine from the mother-liquors of tea-leaves after removal of caffeine, if urea is not added to the nitric acid, nearly one-half of the adenine may be converted into hypoxanthine. By processes of putrefaction adenine is converted into hypoxanthine and guanine into xanthine (Schindler). The change is, therefore, somewhat analogous to that produced by nitrous acid. Adenine undergoes this decomposition much more rapidly than the other xanthine compounds.

The ease with which adenine and guanine are reduced outside of the organism shows that similar changes may take place within the cell-nucleus proper. For we know that every cell is endowed with an enormous reducing power, and hence it is not difficult to see how the oxygen-free adenine can be readily converted into a body or bodies which greedily take up oxygen. We must, therefore, look upon adenine and guanine not only as the antecedents of hypoxanthine and xanthine, but also as intermediate products which, when they form in the cell, may give rise to important chemical processes, especially those of a synthetic nature. It is highly probable that the study of the decomposition-products of nuclein will explain to us many of the metabolic changes in the organism, and throw additional light upon the migration of the amido group from the proteid molecule to the amido acids and urea derivatives. Thus, the formation of xanthine from guanine represents the conversion of a guanidine residue into a urea residue. A similar change is undoubtedly effected in the transformation of adenine into hypoxanthine.

Adenine unites with bases, acids, and salts. The salts of adenine with mineral acids can be recrystallized, thus differing from the corresponding salts of guanine and hypoxanthine, which are dissociated by the action of water. The solutions of the salts, however, show an acid reaction to litmus but not to methyl-orange.

The hydrochloride, $C_5H_5N_5.HCl + \frac{1}{2}H_2O$, forms colorless, transparent, strongly refracting crystals. One part of the anhydrous salt is soluble in 41.9 parts of cold water. Microscopically it is distinct from that of hypoxanthine and adenine-hypoxanthine.

The nitrate, $C_5H_5N_5.HNO_3 + \frac{1}{2}H_2O$, crystallizes from the aqueous solution in fine, stellate needles. One part of the dry salt dissolves in 110.6 parts of water.

The sulphate, $(C_5H_5N_5)_2.H_2SO_4 + 2H_2O$, can be obtained from the aqueous solution in two different crystalline forms. This may possibly be due to the presence of adenine-hypoxanthine compound (Bruhns). It is easily soluble in hot water, and at the ordinary temperature it is soluble in 153 parts of water.

The oxalate, $C_5H_5N_5.C_2H_2O_4 + H_2O$, is obtained by dissolving adenine in hot, dilute, aqueous oxalic acid, from which solution, on cooling, it separates as a voluminous, difficultly soluble precipitate of roundish masses which are composed of long, delicate needles. The oxalates of guanine, hypoxanthine, and xanthine are more easily soluble than that of adenine, and exhibit, moreover, a different appearance.

The picrate, $C_5H_5N_5.C_6H_2(NO_2)_3OH + H_2O$, is thrown down as a bright yellow flocculent precipitate, when aqueous solutions of adenine salts are treated with sodium picrate. Recrystallized from hot water it forms bright-yellow, very voluminous bunches of long fine needles, which, on drying, acquire a silky lustre and form a felted mass. It is difficultly soluble in cold water (1 : 3500); more readily in hot water and in alcohol (96 per cent.); is insoluble in dilute acids. The water of crystallization is not lost on exposure to air but is driven off at 100°; the salt then remains unchanged even at 220°. A cold concentrated aqueous solution of the salt treated with one-tenth its volume of cold concentrated solution of sodium picrate produces a precipitate of short fine needles consisting of most of the adenine picrate (five-sevenths). The solubility of the picrate can thus be reduced to as low as 1 : 13750, and on this fact is based the quantitative method of **Bruhns**. The salt can also be obtained in its characteristic groups by combining cold saturated aqueous adenine solution (1 : 1086) with picric acid; with sodium picrate, however, adenine gives no precipitate, since the picrate is soluble in an equivalent quantity of sodium hydrate. Thus is explained **Kossel**'s statement that adenine forms an easily soluble compound with picric acid. Heated on a platinum foil it burns slowly and leaves considerable carbon residue. The very bright yellow color of the salt serves to distinguish it from most of the other picrates, especially guanine picrate.

The platinochloride, $(C_5H_5N_5.HCl)_2PtCl_4$, crystallizes from dilute aqueous solution in small yellow needles. The concentrated aqueous solution of this salt, when boiled for some time, decomposes, with the separation of a clear, yellow powder, which is but slightly soluble in cold water, and has the composition $C_5H_5N_5.HCl.PtCl_4$.

The aurochloride, on evaporation, yields very characteristic forms.

The silver salt of adenine, $C_5H_4AgN_5$, is formed when silver nitrate is added in molecular proportion to a boiling ammoniacal solution of adenine. An excess of silver nitrate produces, in the cold, the compound $C_5H_3Ag_2N_5 + H_2O$, which is converted slowly in the cold, immediately on warming, into the other salt, according to the equation:

$$2(C_5H_3Ag_2N_5 + H_2O) = 2C_5H_4AgN_5 + Ag_2O + H_2O.$$

Owing to this instability the two compounds are always found together in varying proportion. Both are difficultly soluble in water, and ammonia even at the boiling-point. The precipitation of adenine by an ammonical silver solution is complete, and is therefore available for quantitative estimation.

Adenine silver nitrate, $C_5H_5N_5.AgNO_3$, ($Ag = 35.4$ per cent.), corresponds to the similar hypoxanthine and guanine salts. It is obtained by dissolving the above silver compounds in hot nitric acid; and from this solution, on cooling, it separates in needle-shaped crystals, which are not permanent. This lack of stability, as compared with the permanent hypoxanthine silver nitrate, was first pointed out by KOSSEL, and was thought to be due to loss of nitric acid in washing, and also by heating at 100°. BRUHNS, however, has shown that the acidity of the wash-water is indicated by litmus, but not by methyl-orange, which is not colored red by silver nitrate. The reaction is, therefore, due not to free nitric acid, but to silver nitrate. It would seem that adenine, as well as hypoxanthine, and possibly xanthine, form silver compounds containing one and two molecules of silver nitrate; the greater the quantity of silver nitrate used the higher is the per cent. of silver, *i.e.*, the more of the latter compound is formed. These are very unstable, and are decomposed by dilute nitric acid, more so by water, into silver nitrate and the compound containing one molecule of silver nitrate. We have in this behavior an interesting case of mass-action and chemical equilibrium between adenine, silver nitrate, nitric acid and water. Ammonium hydrate removes the nitric acid from this as easily as from the hypoxanthine compound, and there is formed, according to the composition of the original salt, a varying mixture of $C_5H_4AgN_5$ and $C_5H_3Ag_2N_5 + H_2O$. The solubility in nitric acid is about the same as that of hypoxanthine silver nitrate.

Adenine silver picrate, $C_5H_4AgN_5.C_6H_2(NO_2)_3OH + H_2O$, is obtained as an amorphous voluminous yellow precipitate when silver nitrate is added to a cold aqueous solution of adenine picrate. If the latter solution is previously raised to the boiling-point the precipitate then soon becomes crystalline and rapidly subsides. The adenine can thus be almost wholly removed from solution. The crystalline form loses its water of crystallization at 120°, while the amorphous form does not appreciably decrease in weight and its composition does not appear to be as constant as that of the corresponding hypoxanthine compound. On treatment with ammonium hydrate the picric acid is removed, and adenine silver, $C_5H_4AgN_5$, is left, stained yellow by traces of picric acid.

Adenine-mercury picrate, $(C_5H_4N_5)_2Hg.2C_6H_2(NO_2)_3OH$, can be prepared by treating a hot concentrated aqueous solution of adenine picrate with an excess of sodium picrate and then with mercuric chloride. It forms a yellow granular crystalline precipitate (microscopic needles) which rapidly subsides and increases in quantity as the solution cools. Its composition apparently varies, containing one to two molecules of water, according to the temperature of the solution. One molecule is given off at 100°, and the second at 105°–120°. The latter preparation, then, on exposure to the air, rapidly absorbs one molecule of water. The object of the sodium picrate in the precipitation is to combine with the hydrochloric acid, which is set free. The precipitate produced by mercuric chloride in cold adenine picrate solution shows yellow and white granules, and is not homogeneous. BRUHNS considers it to be a mixture of the adenine-mercury picrate and the compound $C_5H_4N_5Hg_2Cl_3$; if sodium picrate is added, however, the pure adenine-mercury picrate forms, since no hydrochloric acid is set free.

Adenine-mercuric chloride, $C_5H_4N_5HgCl$, is thrown down as a white, finely granular precipitate when a boiling aqueous adenine solution is treated gradually with concentrated mercuric chloride solution. It is formed according to the following reaction:

$$C_5H_5N_5 + HgCl_2 = C_5H_4N_5HgCl + HCl.$$

That free hydrochloric acid forms can be ascertained by methyl orange. Treated with ammonium hydrate the chlorine is removed, and there is formed apparently the compound $C_5H_4N_5HgOH$. If dissolved in warm dilute hydrochloric acid and allowed to crystallize, the double salt $C_5H_5N_5.HCl.HgCl_2 + 2H_2O$ separates in long stellate silky needles.

Another mercury compound, $C_5H_4N_5Hg_2Cl_3$, is obtained when the precipitation takes place in the cold. The precipitate is white, flocculent, and anhydrous. In this reaction, as above, for each adenine molecule an equivalent of hydrochloric acid is set free. This same body is also produced when an adenine solution is boiled with a large excess of mercuric chloride and as little hydrochloric as possible to effect solution. On cooling small stellate needles separate out, which do not lose their weight at 110°. It can also be obtained by boiling the following compounds with water.

When adenine is boiled with a large excess of mercuric chloride and much hydrochloric acid to completely dissolve the precipitate that first forms, there is deposited on cooling a crystalline product, which is variable in its composition, and apparently consists of double salts of adenine and mercuric chloride, such as $C_5H_5N_5.HCl.5HgCl_2$ and $C_5H_5N_5.HCl.6HgCl_2$. On boiling with water these rapidly decompose, forming the compound $C_5H_4N_5.Hg_2Cl_3$. The formation of a double salt, $C_5H_5N_5.HCl.HgCl_2 + 2H_2O$ is described above.

Adenine-mercury cyanide, $(C_5H_5N_5)_2Hg(CN)_2$, separates as stellate needles and plates when a mixture of hot solutions of adenine and mercuric cyanide are allowed to cool.

An adenine bismuth iodide, $C_5H_5N_5.HI.2BiI_3 + 2H_2O$, is obtained when an aqueous adenine solution is treated with potassium bismuth iodide containing free hydriodic acid. The heavy precipitate, which in color resembles carbon monoxide hæmoglobin, consists of microscopic glittering red needles. On contact with much water it partly decomposes, forming light reddish-yellow amorphous floccules, which become darkish-brown at 100°.

Adenine bromide. By treating well-dried adenine with excess of dried bromine a dark-red body is obtained which appears to contain six atoms of bromine. On mere exposure to the air, more rapidly on heating at 100°–120°, it decomposes, yielding bromine and a brom-adenine, $C_5H_4BrN_5$. This compound is white, difficultly soluble in cold water (1 : 10,000), more readily in hot water, very easily in ammonia. It crystallizes from water or dilute ammonia in stellate needles. It is a rather strong base and forms well-characterized salts from which it is thrown down as a white micro-crystalline precipitate by addition of ammonia. It is also formed from adenine-bromide by treatment with sodium bisulphite. The picrate resembles that of adenine but is more voluminous; silver compounds are also formed resembling those of adenine. The silver nitrate compound decomposes on boiling with nitric acid with separation of silver bromide. It is only difficultly attacked by boiling alcoholic potash.

When adenine is treated with zinc and hydrochloric acid, in the cold, it forms a difficultly soluble crystalline double salt which has not been obtained in the pure state. This double salt is not obtained by direct treatment of adenine hydrochloride with zinc chloride.

One of the hydrogen atoms of adenine is capable of replacement by organic radicals. Thus it forms crystalline methyl and ethyl compounds.

Acetyl-adenine, $C_5H_4N_5.CO.CH_3$, can be obtained by heating the anhydrous base with an excess of acetic anhydride for some time, in an oil-bath, at 130°. It crystallizes in small white scales which dissolve but slightly in cold water and in alcohol; more readily in hot water, in dilute acids and alkalies. Heated to 260° it becomes yellow but does not melt.

Benzoyl-adenine, $C_5H_4N_5.CO.C_6H_5$, is obtained by the action of benzoic anhydride, but not of benzoyl chloride, on adenine. It crystallizes from water in long, lustrous, thin needles which sometimes are grouped in bundles, and melt at 234°–235°. It is easily soluble in hot alcohol, from which it recrystallizes on cooling; also in dilute acids and in ammonia. With ammoniacal silver nitrate it gives a precipitate resembling that of adenine, but is more readily soluble in ammonia. This compound is quite stable, since it decomposes very slowly on boiling with hydrochloric acid; on protracted boiling with water it is changed into adenine and benzoic acid.

Benzyl-adenine, $C_5H_4N_5.CH_2.C_6H_5$, was obtained by THOISS by heating well-dried adenine with benzyl chloride to boiling (178°) on an oil-bath. The compound forms pure white microscopic crystals and melts at 259°. It is easily soluble in hot water and in hot alcohol. With acids it forms salts from which alkalies throw down the base. The hydrochloride forms fine glossy needles which are readily soluble in alcohol and in water, but not in ether. The sulphate and nitrate possess similar properties. Like adenine it yields a silver compound which is insoluble in ammonia. On reduction with zinc and hydrochloric acid it forms an amorphous red unstable compound. Treated with nitrous acid, benzyl-adenine is reduced to benzyl-hypoxanthine, thus showing that the benzyl group replaces a hydrogen atom in the group $C_5H_4N_4$, which KOSSEL has called adenyl (see page 307).

Benzyl-adenine picrate, $C_{12}H_{11}N_5.C_6H_2(NO_2)_3OH$, is obtained as fine felted yellow needles, which are fairly soluble in water and in alcohol; insoluble in ether.

A methyl-adenine was obtained by THOISS in an impure state by heating dried adenine with methyl iodide in a sealed tube at 100°. It can be crystallized from absolute alcohol. The aqueous solution of the base is precipitated by baryta water; alcoholic zinc chloride also yields a precipitate which is soluble in excess of ammonium hydrate. Mercuric nitrate also gives a precipitate. Cadmium chloride yields a precipitate which dissolves on warming, reappears on cooling, and is soluble in ammonia. Basic lead acetate has no effect.

Nothing definite is known in regard to the physiological action of adenine, except that when fed to dogs it appears to be eliminated as such, in part at least, by the urine.

ADENINE-HYPOXANTHINE, $C_5H_5N_5 + C_5H_4N_4O$. The occurrence of this compound was observed by KOSSEL, but it was isolated and studied for the first time by BRUHNS. It can be prepared by cooling a hot aqueous solution of equal parts of the two bases. At first it is obtained as thick, starch-like semi-transparent masses, which later in part become white and chalky. By spontaneous evaporation of its solution in very dilute ammonia it forms pearly aggregates of very small radially arranged needles, which contain water of crystallization. These effloresce somewhat and lose the water at 100°. The compound is more readily soluble in water than its components, but an exact determination of its solubility is impossible, inasmuch as the separation from hot solutions is not completed for some weeks. Any adenine present can be separated by recrystallization. It forms a distinct crystalline hydrochloride, which should be borne in mind when examining microscopically for the two bases; but the combination is loose, since addition of gold chloride brings down the characteristic gold salt of adenine. Ordinarily it does not form salts with sulphuric or nitric acids, but more often is decomposed by these, so that the difficultly soluble adenine crystallizes out. Once, however, BRUHNS obtained a sulphate which differed from the pure adenine and hypoxanthine sulphates; thus is perhaps explained the observation of KOSSEL that adenine sulphate forms crystals belonging to two systems. The compound can be decomposed into its constituents by fractional crystallization of the sulphate or nitrate; but better by forming the picrates, which are very unequally soluble in water. The existence of this compound undoubtedly explains many of the mistakes and discrepancies concerning the properties of hypoxanthine, which it resembles more than adenine, and for the same reason, perhaps, adenine was so often overlooked.

HYPOXANTHINE, $C_5H_4N_4O$, sometimes also known as sarcine or sarkine, was discovered by SCHERER (1850) in splenic pulp and in the muscles of the heart, and was named thus because it contains one atom of oxygen less than xanthine. It has since been obtained, usually accompanying adenine and guanine, from nearly all of the animal tissues and organs rich in nucleated cells, *i. e.*, in nuclein. It has been found in blood after death, but not in blood when flowing through the bloodvessels. SALOMON has recently shown it to be a normal constituent of urine, present, however, in an exceedingly minute quantity. In the blood and urine of leucocythæmic patients it occurs in increased quantity owing to the abnormally large number of nucleated white blood-corpuscles in circulation (see page 284). BENCE JONES observed in the urine of a boy, who about three years before showed the symptoms of renal colic, a deposit of characteristic whetstone-like crystals, resembling uric acid, but differing from the latter by dissolving readily on the application of heat, while from hydrochloric acid it crystallized in elongated six-sided plates. These crystals he believed to be those of xanthine, but SCHERER and others consider them to be hypoxanthine. It is therefore quite possible, though very rare, for this base to form a deposit in the urine and to be confounded in shape with uric acid. THUDICHUM has obtained it from the urine of persons sick with liver or kidney diseases.

Among other places it has been found in the brain, muscle, serum, marrow of bones, kidney, heart, spleen, liver, peripheral muscles (sarkine of STRECKER); in the spawn of salmon (PICCARD), in the testicles of the bull (SALOMON), in the nuclein of pus and red corpuscles (KOSSEL), in developing eggs, and in putrefaction of albumin (SALOMON). It has also been found in the spores of lycopodium, and in the pollen of various plants, in seed of black pepper, in grass, clover, oats, bran of wheat, larvæ of ants; in the juice of potato (SCHULZE); in certain wines (KAYSER); in the aqueous decoction of yeast of beer (SCHÜTZENBERGER); and also in the liquid in which yeast is grown (BECHAMP). DEMANT has shown it to be relatively abundant in the muscles of pigeons in a state of inanition, while in muscles of well-fed pigeons it is said to be entirely absent. SALOMON found hypoxanthine and xanthine in the cotyledons of lupine, as well as in the sprouts of malt, while REINKE and RODEWALD observed these two bases together with guanine in Æthalium septicum—with adenine, xanthine, and theophylline, it occurs in tea-leaves (KOSSEL).

Hypoxanthine has not been extracted from the pancreas, where it seems to be replaced by guanine, or rather by adenine. It seems that hypoxanthine bears a relation to adenine similar to that which we see between glycocoll and glycocollic acid.

Hypoxanthine occurs frequently in plants together with the other members of this group, namely, adenine, guanine, and xanthine. The widely distributed character of these bases is due to the presence of a parent substance, viz., nuclein, the necessary constituent of all cells capable of development, which under the influence of acids, and probably likewise of ferments, decomposes into the above-mentioned bases. They may, therefore, be considered as the first steps in the retrograde metamorphosis of all tissues, since they have their origin in nuclein, an important proteid substance. Recent advances in biological chemistry have shown that the undeveloped eggs of various insects and birds yield much less quantity of xanthine bodies (hypoxanthine, xanthine, etc.) on treatment with dilute acid than the partially developed eggs (TICHOMIROFF, KOSSEL). This is dependent upon the remarkable fact observed by KOSSEL that the nuclein of undeveloped chicken eggs differs from the nuclein of cell nuclei and resembles that obtained from milk. For, while the nuclein from the cell nuclei decomposes into adenine, guanine, hypoxanthine, etc., that from undeveloped eggs and from milk yields no nitrogenous bases on treatment with acids. But as the egg develops, *i.e.*, the nucleated cells increase in number, this latter nuclein is gradually converted or gives way to the ordinary cell nuclein, and hence it is that the chick embryo yields guanine, hypoxanthine, and possibly adenine.

Unquestionably, the presence of hypoxanthine, etc., in developing cells is due to the presence of the nuclein molecule, from which it is readily split off. In muscle, however, hypoxanthine and xanthine appear to exist preformed, and bear no relation to nuclein, since they are in the free condition, and can be extracted from the tissue by water. For an explanation of this peculiar fact, see Adenine, page 284, and Guanine, page 308.

According to the observations of SALOMON and CHITTENDEN, hypoxanthine is formed by the digestion of blood fibrin with gastric juice, pancreatic juice, or on heating with water or dilute acids. Egg albumin under the same conditions does not yield any hypoxanthine, except when treated with pancreatic juice. These observations require repetition, inasmuch as the fibrin used undoubtedly contained nuclein, which, as we now know, readily decomposes under those conditions into its characteristic nitrogenous bases. Be that as it may, it appears, however, to be one of the products formed by the decomposition and successive oxidation of proteid matter previous to the formation of uric acid and urea.

When a mixture of guanine, xanthine, and hypoxanthine is allowed to putrefy, the bases decompose and disappear in the order named. Hypoxanthine resists bacterial action the longest, and this corresponds with its behavior to reagents (BAGINSKY). Adenine during putrefaction, in the absence of air, is converted into hypoxanthine, and guanine is correspondingly changed into xanthine (SCHINDLER). An imido group is, therefore, replaced by oxygen, and probably goes to form urea. This conversion is a very important fact, since the process of putrefaction, as HOPPE-SEYLER has repeatedly pointed out, is analogous to the vital process, and the same chemical change may take place in the animal organs. The same change very probably takes place in the auto-digestion of yeast. Its formation under these conditions can be represented thus:

$$C_5H_5N_5 + H_2O = C_5H_4N_4O + NH_3.$$

Hypoxanthine can be readily obtained from a number of closely related substances. Thus, carnine, by the action of oxidizing agents, is converted into hypoxanthine (page 328). For this reason WEIDEL and SCHÜTZENBERGER regard hypoxanthine as derived from carnine, but this view is now entirely set aside by our present knowledge of the relation of this base to nuclein.

Again, it can be obtained from adenine (page 289) by the action of nitrous acid. The relation that hypoxanthine bears to uric acid is shown by the fact that the latter is converted by nascent hydrogen first into xanthine, and finally into hypoxanthine.

$$\underset{\text{URIC ACID.}}{C_5H_4N_4O_3} + 2H_2 = C_5H_4N_4O + 2H_2O.$$

This transformation of uric acid into hypoxanthine is of especial importance, since together with HORBACZEWSKI's synthesis of uric acid, accomplished by acting on urea with either glycocoll or trichlorlactamide, it constitutes the last step in the complete synthesis of hypoxanthine from its elements.

Hypoxanthine has been hitherto regarded as a step lower than guanine in the series of nitrogenous products of regressive metamorphosis, and consequently was considered as derived from guanine. The investigations of KOSSEL, however, show that it arises not from guanine but from adenine. On the other hand, guanine is to be looked upon as the source of xanthine. It is probable that in the organism it is oxidized as soon as it is set free from the nuclein, forming successively xanthine, uric acid, urea, etc., and the small quantity present in the urine is all that has escaped oxidation. When fed to dogs, it was observed that the amount of hypoxanthine present in the urine decreased, and even became less in amount than before the experiment; but, on the other hand, the amount of xanthine in the urine was found to have been increased above the normal. This shows that hypoxanthine in the body is oxidized probably first to xanthine, then into uric acid. According to ROBERT hypoxanthine is a true muscle stimulant.

The fact that hypoxanthine is so widely distributed in the organism, and in much larger quantities than was formerly supposed, shows that it constitutes, together with the closely related bodies creatine, xanthine, guanine, etc., the normal antecedents of urea and uric acid. This view is furthermore strengthened since hypoxanthine is especially abundant in those organs which are most active in producing metabolic changes in the body, viz., the liver and spleen.

It may be prepared from the urine, according to the method given under paraxanthine (page 322); or from extract of meat, or from glandular organs, such as the liver, spleen, etc., by the process on page 285. Nuclein, on decomposition with acids, yields about one per cent. of this base. It can be determined with adenine indirectly by SCHINDLER'S method (page 286); but better still directly by BRUHN'S picrate method (see page 286). After the adenine has been precipitated by sodium picrate, the determination of hypoxanthine in the filtrate is not difficult if hydrochloric and other acids, the silver salts of which do not quite dissolve in ammonia, are absent. The filtrate from the adenine picrate is rendered slightly alkaline with ammonia and precipitated with silver nitrate at the boiling-point. The slightly yellow-colored precipitate is washed with hot water till the wash-water is colorless; then dried at 120° for from two to three hours, when it has the composition $2C_5H_2Ag_2N_4O + H_2O$. It contains, however, traces of picric acid and some adenine silver, and hence the quantity of hypoxanthine calculated from the weight obtained is higher than it really is. BRUHNS, as a correction, subtracts 3.0 mg. from the calculated quantity of hypoxanthine.

A more convenient method than the preceding is to estimate hypoxanthine as hypoxanthine silver picrate. The filtrate from the adenine picrate (page 287) is raised to the boiling-point and silver nitrate solution gradually added. The precipitate is washed with cold water till the wash-water is colorless, then dried at 100°, when its composition is represented by the formula $C_5H_3AgN_4O.C_6H_2(NO_2)_3OH$. The calculated quantity of hypoxanthine here is likewise slightly higher than it should be. Bruhns deducts 1.0 mg. from the calculated result.

In the presence of hydrochloric acid, etc., the determination of hypoxanthine is somewhat circuitous since the precipitated silver chloride must be separated from the hypoxanthine compound. The best procedure in this case is to saturate the filtrate from adenine picrate with ammonia and precipitate it completely with silver nitrate. The precipitate is washed with hot water (a thorough washing is not necessary), then it is boiled several times with nitric acid of 1.1 specific gravity. The acid each time is rapidly decanted on to a small filter, and finally the residue washed on the filter with 10 c.c. of the hot acid (total 100 c.c.). To the combined acid filtrate silver nitrate is added, and the whole set aside for twenty-four hours. The precipitate is dried at 100°. The amount of hypoxanthine lost depends upon the quantity of silver chloride present. The correction to be added is 3.1 mg. (Bruhns). In Neubauer-Kossel's method the mixed adenine and hypoxanthine silver salts can be decomposed with a little hydrochloric acid and estimated in this way.

Hypoxanthine is a white, colorless, crystalline powder, sometimes in part amorphous; according to Bruhns, pure hypoxanthine does not form floccules and bunches of microscopic needles, but usually coherent crusts, which consist of roundish, sharp-cornered granules; some resemble quadratic octahedra. It is soluble in about 300 parts of cold water (Strecker). The base separates slowly from aqueous solutions, and when pure the solubility, even in the beginning, is less than 1 : 300. At the end of four days Bruhns found it to be 1 : 1880. It is more easily soluble in boiling water [illegible] n cooling, separates in the form of whit [illegible] thus differing from xanthine, which is amorphous. The solubility in cold alcohol is very slight, about 1 : 1000. It dissolves in acids and alkalies without decomposition, and from solutions in the latter it can be precipitated by passing carbonic acid, or by the addition of acetic acid. The aqueous solution possesses a neutral reaction. The free base can be heated up to 150° without suffering decomposition, but above this temperature it sublimes, and partially decomposes, with evolution of hydrocyanic acid. When heated with potassium hydrate to 200°, it yields ammonia and potassium cyanide. Heated with water to 200°, it decomposes into carbonic acid, formic acid, and ammonia, and in this respect it agrees with adenine (page 288). The properties of STRECKER'S sarkine agree closely with those of adenine-hypoxanthine; and, inasmuch as the latter has been often described as hypoxanthine, it is very desirable that the properties of hypoxanthine be redetermined.

When evaporated with an oxidizing agent, chlorine water or nitric acid, the residue is said to give on contact with ammonia vapors a rose-red color (WEIDEL, murexide test). KOSSEL, however, has shown that this is due to the presence of xanthine, and that pure hypoxanthine does not give either the murexide test or the xanthine reaction. According to STRECKER, concentrated nitric acid converts hypoxanthine into a nitro-compound, which in turn, by the action of a reducing agent, is changed into xanthine. This statement has not been confirmed either by FISCHER or by KOSSEL. It does not give a green color with sodium hydrate and chloride of lime—distinction from xanthine (page 316).

With acids it yields crystallizable compounds, and, like the amido acids, it forms compounds with bases, and also with metallic salts, such as silver nitrate and copper acetate.

The hydrochloride, $C_5H_4N_4O.HCl + H_2O$, crystallizes in needles, and, like the nitrate and sulphate, it is dissociated on contact with water. The crystalline form is characteristic and distinct from that of adenine, as well as adenine-hypoxanthine. The nitrate forms thick prisms or roundish masses, readily soluble in water and ammonia. Platinum chloride forms a yellow, crystalline double salt, having the composition $C_5H_4N_4O.HCl.PtCl_4$.

The picrate forms yellow prisms easily soluble in water, which solution is not affected as that of adenine by sodium picrate.

Hypoxanthine silver, $C_5H_2Ag_2N_4O.H_2O$. All attempts to obtain a compound containing but one atom of silver in the molecule, corresponding to the adenine compound $C_5H_4AgN_5$, have failed. The above compound was first prepared by STRECKER, and given the formula $C_5H_4N_4O.Ag_2O$; but the former is preferable, since on heating at 120° two and a half molecules of water are lost and

$$2C_5H_2Ag_2N_4O + H_2O \text{ (Ag} = 60.2 \text{ per cent.) results.}$$

At 140°–150° it loses again in weight and becomes gradually gray; on exposure to air it absorbs moisture. In this form hypoxanthine can be estimated quantitatively (see page 302); the presence of sodium picrate does not interfere, but chlorides, etc., do. It is insoluble in hot water. The compound, $C_5H_2Ag_2N_4O.3H_2O$, is obtained in the form of microscopic needles, by treating pure hypoxanthine silver nitrate with excess of aqueous ammonia. On boiling with ammonia-water it is but slightly dissolved, and appears to slowly lose a part of its water of crystallization. As a result of the decomposition one-half of the hypoxanthine passes into solution and can be recovered on boiling with addition of silver nitrate in the crystalline form; or in the cold, as the usual amorphous precipitate, $C_5H_2Ag_2N_4O.H_2O$.

Hypoxanthine silver nitrate, $C_5H_4N_4O.AgNO_3$, ($Ag =$ 35.29 per cent.), is the best-known compound; its formula was established by STRECKER. It is obtained by dissolving the above precipitate, produced by addition of silver nitrate to an ammoniacal solution of the base, in hot nitric acid, specific gravity 1.1; on cooling the hypoxanthine silver nitrate crystallizes in the form of tufts of microscopic needles or plates. Heated at 100°–120° it remains constant in weight; the quantity of silver present, when determined, is always somewhat higher than the theoretical, especially if an excess of silver nitrate is employed in the precipitation. The explanation of this fact is probably that given under Adenine, though presence of silver chloride may partly be the cause. On treatment with ammonia it loses not only nitric acid but also half of the hypoxanthine, and $C_5H_2Ag_2N_4O.3H_2O$ forms. The change takes place readily even in the cold, and if during the digestion an excess of silver nitrate is added, the hypoxanthine set free is converted into this compound, which is wholly constant in composition compared with the hypoxanthine silver nitrate. The conversion is quantitative. Very dilute hydrochloric acid, as well as hydrogen sulphide, removes the silver from this compound.

Hypoxanthine-silver picrate,

$$C_5H_3AgN_4O.C_6H_2(NO_2)_3OH \text{ (Ag} = 22.88 \text{ per cent.),}$$

is gradually formed by adding silver nitrate to a boiling solution of hypoxanthine picrate. The precipitate is granular and of a lemon-yellow color, and consists of aggregations of fine short needles. It is slightly soluble in hot, insoluble in cold water. It is, therefore, applicable for a quantitative determination of the base. Aqueous ammonia very readily and completely removes the picric acid from the compound, and the residue is hypoxanthine silver, which is slightly colored yellow by a trace of picric acid; half of the hypoxanthine passes into solution. Nitric acid with difficulty converts it into hypoxanthine silver nitrate.

Hypoxanthine mercuric chloride, $C_5H_3N_4OHgCl$, is obtained by adding an equivalent quantity of mercuric chloride to a boiling solution of hypoxanthine. The precipitate, which increases on cooling, is crystalline.

A second compound, $C_5H_3N_4OHg_2Cl_3$, is produced by adding a strong excess of mercuric chloride, in the cold, to an aqueous solution of hypoxanthine. It forms a heavy granular micro-crystalline precipitate, which contains some water of crystallization.

By boiling the preceding compound with just sufficient hydrochloric acid to effect complete solution, there is formed on standing a precipitate of white roundish aggregates of leafy or needle-shaped glittering crystals which have the composition $C_5H_4N_4OHgCl_2 + H_2O$.

The following table of **Bruhns** illustrates the analogy existing between the mercury compounds of adenine and hypoxanthine and similar derivatives of ammonium:

AMMONIUM.	ADENINE.	HYPOXANTHINE.
NH_2HgCl	$C_5H_4N_5HgCl$	$C_5H_3N_4OHgCl(+H_2O)$
$NH_2Hg_2Cl_3$	$C_5H_4N_5Hg_2Cl_3$	$C_5H_3N_4OHg_2Cl_3(+H_2O)$
$(NH_3)_2HgCl_2$	$(C_5H_5N_5)_2Hg(C_n)_2$ $(C_5H_5N_5)_2Hg.2C_6H_2(NO_2)_3OH$	$C_5H_4N_4OHgCl_2(+H_2O)$

A brom-hypoxanthine compound corresponding to that of adenine has not been obtained.

Benzyl-hypoxanthine, $C_5H_3N_4O.CH_2.C_6H_5$, was obtained by **Thoiss** by the action of nitrous acid on benzyl-adenine. It forms a white crystalline mass which under the microscope consists of thin plates. It is easily soluble in hot water, dilute alcohol, and in acetic ether; insoluble in ether and chloroform. It melts at 280°. It appears, as **Kossel** has pointed out, that adenine and hypoxanthine contain a group, $C_5H_4N_4$, which he named *adenyl*. The formation of the benzyl derivatives of these two bases shows that the hydrogen atom which is replaced occurs in the adenyl and not in the imido group. According to this view adenine is to be considered as adenylimide ($C_5H_4N_4.NH$) and hypoxanthine as adenyloxide ($C_5H_4N_4O$).

Phosphomolybdic acid precipitates hypoxanthine from acid solution, and in general it gives the ordinary alkaloidal reactions.

It is not precipitated by ammoniacal basic lead acetate. Copper acetate does not precipitate it in the cold, but does on boiling. This fact has been made use of in the isolation of hypoxanthine. Mercuric chloride, as well as mercuric nitrate, produces a flocculent precipitate.

Altogether, in its behavior to reagents it resembles xanthine to a very considerable degree. The two can be separated, however, by the different solubilities of the hydrochlorides in water, and more especially of the silver salt in nitric acid.

Physiological Action.—25–100 mg. begin to act on frogs in from six to twenty-four hours, and produce increased reflex excitability and convulsive attacks; 5–100 mg. is fatal (Filehne). When injected subcutaneously into hepatotomized geese or chickens a corresponding increase in uric acid secretion is observed (v. Mach). This conversion is analogous to that observed by Stadthagen in the case of guanine (page 310), and shows that in the xanthine bodies we have antecedents of uric acid apart from the synthesis of the latter from ammonia in the liver. The process by which this change is effected is undoubtedly one of oxidation.

Guanine, $C_5H_5N_5O$, was discovered, in 1844, by Unger, as a constituent of guano, in which it is present in varying quantities according to the region from which the guano comes. Thus, the Peruvian guano is reported as containing the largest proportion of this base, and on that account this variety is employed when it is desired to prepare guanine. Since its discovery by Unger, it has been met with in a very large number of tissues, both animal and vegetable; in the liver, pancreas, lungs, retina, in the thymus gland of the calf, and in the testicle substance of the bull; in the scales of the bleak, and in the swimming-bladder of fish, as well as in the excrements of birds, of insects, as the garden spider, in which it occurs with a small quantity of uric acid (Weinmann), and is to be regarded as a decomposition product of proteids formed in the tissues of the spider. It is also found in the spawn and testicle of salmon, and Schulze and others have shown it to be present in the young leaves of the plane-tree, of vine, etc., also in grass, clover, oats, as well as in the pollen of various plants. Schützenberger has isolated it, together with hypoxanthine, xanthine, and carnine, from yeast which had been allowed to stand in contact with water at near the body-temperature. Pathologically, it occurs in the muscles, ligaments, and joints of swine suffering from the disease known as guanine-gout. Normally, guanine, like adenine, is present in muscle tissue only in traces. It has never been found in the urine, though xanthine has been mistaken for guanine by some.

As to the origin of this substance in the organism very little has been known up to within a few years, except so far as it has been shown to be, together with other members of this group, a transitory product in the retrograde metamorphosis of nitrogenous foods and tissues. In the case of the lower animals it is evidently the end-product of all change, inasmuch as it is excreted as such. Our knowledge as to the immediate origin of this and the other allied bases has lately been extended by the brilliant researches of Kossel on the decomposition products of nuclein, in which he has shown that this essential constituent of all nucleated cells, whether animal or vegetable, decomposes under the action of water or dilute acids into adenine, guanine, hypoxanthine, and xanthine. We know that the first two bases are readily converted by the action of nitrous acid into the other two; that is to say, an NH group in these bases is replaced by an atom of O—a change which it is not at all unlikely takes place in the tissues, perhaps in every cell nucleus. That such a change is quite probable is shown by the putrefaction experiments of Schindler, whereby adenine and guanine were converted respectively into hypoxanthine and xanthine. If this explanation is correct, then adenine and guanine are transition-products between the complex proteïd molecule on the one hand, and hypoxanthine and xanthine on the other. These two, in turn, form the connecting link to the last step in the regressive metamorphosis of the nitrogenous elements of the tissues, viz., the formation of uric acid and urea. We can thus trace a succession of cycles from the complex nuclein molecule, which is apparently indispensable to the functional activity of all reproductable cells, to the physiologically waste products urea and uric acid.

Schulze and Bosshard recently (1886) found in young vetch, clover, ergot, etc., a new base, to which they have given the name vernine. It has the formula $C_{16}H_{20}N_8O_8$, and is of especial interest at this point, since on heating with hydrochloric acid it apparently yields guanine. We have, therefore, at least two well-defined sources of guanine, the nucleins and vernine.

Neither adenine nor guanine occur in normal muscle further than in mere traces, a fact which can only be explained on the ground that the muscle tissue is poor in nucleated cells, and hence in nuclein. Just as the muscle cell has become morphologically differentiated from the typical cell, it may be looked upon also as having undergone a concomitant chemical differentiation, inasmuch as we no longer find the phosphoric acid, xanthine, and hypoxanthine in the same chemical combination as they occur in the original cell. The phosphoric acid, instead of existing as a part of an organic compound, is present in the muscle tissue as a salt; similarly the hypoxanthine and xanthine occur in the free condition, extractable by water, and no longer in combination with other groups of atoms constituting a part of a more complex molecule—nuclein.

Guanine and creatine apparently mutually replace one another. Thus, in the muscle, as just stated, guanine occurs only in traces, whereas creatine is especially abundant. This may find its explanation in the fact that both are substituted guanidines. Creatine is regarded by HOPPE-SEYLER as an intermediate product in the formation of urea, and a similar rôle, it will be remembered, belongs to guanine. From STADTHAGEN'S experiments on dogs we know that guanine ingested, produces an increase in the amount of uric acid and urea excreted, and the same is also true of the nuclein derived from yeast. These results have led him to the conclusion that in mammals uric acid is a direct, or more or less altered cleavage product of proteids, notwithstanding the fact that in birds it is the result of synthesis in the liver.

In the decomposition of nuclein-containing substances, such as yeast, liver, spleen, etc., by dilute acids, neither adenine nor guanine is found alone, but they are always accompanied by hypoxanthine, and usually by a very small quantity of xanthine.

Guanine may be readily prepared from Peruvian guano by boiling it repeatedly with milk of lime until the liquid becomes colorless. The residue, consisting largely of uric acid and guanine, is boiled with a solution of sodium carbonate, filtered, and the filtrate, after the addition of sodium acetate, is strongly acidulated with hydrochloric acid. This precipitates the guanine, together with some uric acid. The precipitate is dissolved in boiling hydrochloric acid, and the guanine then thrown out of solution by the addition of ammonium hydrate. Guanine is also obtained in the decomposition of nuclein with dilute acids, and can, therefore, be prepared from such cellular organs as the spleen, pancreas, etc., according to the method given on page 285. It should be noted here that in the decomposition of the mixed silver compounds with hydrogen sulphide or ammonium sulphide (SCHINDLER) the guanine, often only in part, passes into solution with adenine and hypoxanthine, and the remainder is held back in the silver sulphide precipitate. The latter should, therefore, be boiled with dilute hydrochloric acid, and on saturating the filtrate with ammonia the guanine after a while separates. That portion of the guanine which did pass into solution with the other two bases is separated from them by digestion with ammonia on a water-bath. The two portions are then combined, transferred to a filter, previously dried at 110° and weighed, washed well with ammonia, then dried and weighed.

The free base forms a white, amorphous powder, insoluble in water, alcohol, ether, and ammonium hydrate; easily soluble in mineral acids, fixed alkalies, and in excess of concentrated ammonium hydrate. It can be heated to above 200° without undergoing decomposition. When evaporated with strong nitric acid it gives a yellow residue, and this on the addition of sodium hydrate assumes a red color, which on heating becomes purple, then indigo-blue; on cooling it returns to a yellow, passing through purple and reddish-yellow shades due, according to v. BRÜCKE, to absorption of water. This is the so-called xanthine reaction, and is supposed to be due to the formation of xanthine and a nitro product. It is given best by guanine, then by xanthine, and is not given by either hypoxanthine or adenine.

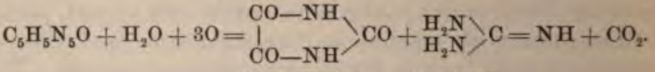
Nitrous acid converts it directly into xanthine, thus:

$$C_5H_5N_5O + HNO_2 = C_5H_4N_4O_2 + N_2 + H_2O.$$

This reaction is identical with that of adenine, whereby hypoxanthine is formed (see page 289). By putrefaction in the absence of air it forms xanthine (SCHINDLER). The change can be represented by the equation:

$$C_5H_5N_5O + H_2O = C_5H_4N_4O_2 + NH_3.$$

On oxidation with potassium permanganate it yields urea, oxalic acid, and oxy-guanine. By hydrochloric acid and potassium chlorate it is oxidized to carbonic acid, guanidine, and parabanic acid, according to the equation:



$$C_5H_5N_5O + H_2O + 3O = \begin{matrix} CO-NH \\ | \\ CO-NH \end{matrix}\!\!>CO + \begin{matrix} H_2N \\ H_2N \end{matrix}\!\!>C = NH + CO_2.$$

PARABANIC ACID. GUANIDINE.

According to STRECKER, a small amount of xanthine is formed in this reaction, and it is quite possible that this base is also formed on oxidation with nitric acid.

Guanine combines with acids, bases, and salts. It unites with bases to form crystalline compounds; and with one or two equivalents of acid it also yields crystallizable salts. Thus, with hydrochloric acid it forms the two salts, $C_5H_5N_5O.(HCl)_2$ and $C_5H_5N_5O.HCl + H_2O$. Similar combinations can be obtained with nitric acid. The sulphate $(C_5H_5N_5O)_2H_2SO_4$, crystallizes in long needles, and, like the other salts, is decomposable by water. The platinochloride, $(C_5H_5N_5O.HCl)_2PtCl_4 + 2H_2O$, is readily obtained in a crystalline condition. The silver compound is soluble in hot nitric acid, and on cooling recrystallizes in fine, needle-shaped crystals, having the composition $C_5H_5N_5O.AgNO_3$.

The solutions of the hydrochloride are precipitated by mercuric chloride and nitrate, potassium chromate, potassium ferricyanide, and by picric acid. Basic lead acetate gives a precipitate only on addition of ammonium hydrate. The reaction with picric acid (CAPRANICA) is said to be very characteristic, and a means of distinguishing this base from xanthine and hypoxanthine. It is best obtained by adding a cold, saturated solution of picric acid to the warm acidulated solution of guanine, when a light, crystalline precipitate forms. Under the microscope it appears in pencil-shaped, fern-like tufts of fine, orange-yellow needles.

Physiologically guanine like uric acid is inert (**Filehne**).

Xanthine, $C_5H_4N_4O_2$, is also very widely distributed in the organism, and has been met with in almost all the tissues and liquids of the animal economy. Together with hypoxanthine, guanine, and possibly adenine, it occurs in many plants, among which may be mentioned lupine, æthalium, sprouts of malt, tea-leaves (**Baginsky**), auto-digestion of yeast, gourd seeds, soja beans, etc. It was first discovered by **Marcet** (1819) in a urinary calculus, and since then has been frequently found as the only or chief constituent of many calculi. **Unger** and **Phipson** have extracted it from guano, while **Salomon** has shown it to be one of the products formed in the pancreatic digestion of fibrin. **Schützenberger** found it together with carnine and hypoxanthine in the liquors from yeast. It is a normal constituent of the urine, but is present only in extremely minute quantities. During the use of sulphur-baths, or after the thorough application of sulphur salves, the quantity of xanthine in the urine is considerably increased. It is likewise more abundant in the urine of leucocythæmic patients, for the reasons already given on page 283. **Baginski** holds that the amount of xanthine normally present in the urine may be increased tenfold in the case of acute nephritis. **Bence Jones** observed in the urine of a child sick with renal colic, a deposit of crystals which he considered to be xanthine, but other observers are inclined to regard the crystals as those of hypoxanthine. **Vaughan** has reported the presence of xanthine in deposits from the urine of patients with enlarged spleen.

Xanthine may be prepared synthetically in several ways. Thus, it may be obtained by the reduction of uric acid by means of sodium amalgam, according to the equation :

$$\underset{\text{Uric Acid.}}{C_5H_4N_4O_3} + H_2 = \underset{\text{Xanthine.}}{C_5H_4N_4O_2} + H_2O.$$

Now that uric acid has been prepared synthetically, this forms the final step in the complete synthesis of xanthine. By further action of nascent hydrogen the xanthine in turn is converted into hypoxanthine. The reverse operation, the conversion of hypoxanthine into xanthine, though reported by STRECKER has not been confirmed by FISCHER or by KOSSEL. It is, therefore, evident that these bodies form a continuous oxidation series with uric acid as the final product. Although this change is unquestionably the one which goes on in the animal economy, yet all attempts to reproduce it in the laboratory by oxidation with potassium permanganate or nitric acid have apparently yielded only negative results. Again, xanthine may be prepared from guanine by putrefaction of the latter, or by oxidation with nitrous acid. The change may be represented by this equation:

$$\underset{\text{GUANINE.}}{C_5H_5N_5O} + HNO_2 = \underset{\text{XANTHINE.}}{C_5H_4N_4O_2} + N_2 + H_2O.$$

This reaction, first described by STRECKER (1858), corresponds exactly to the one by which KOSSEL has transformed adenine into hypoxanthine (see page 289).

GAUTIER, starting out on the hypothesis that xanthine is a polymerization-product of hydrocyanic acid, has endeavored to prepare it directly from this compound. Indeed, he claims to have succeeded in effecting the synthesis of not only xanthine, but also its homologue, by simply heating hydrocyanic acid in a sealed tube with water and a little acetic acid, the latter being added to neutralize any ammonia that might form. He expresses the reaction as follows:

$$11HCN + 4H_2O = \underset{\text{XANTHINE.}}{C_5H_4N_4O_2} + \underset{\text{METHYL-XANTHINE.}}{C_6H_6N_4O_2} + 3NH_3.$$

Nearly all of the methods that have been employed for the preparation of xanthine are based upon its precipitation as the insoluble silver compound. From the urine it can be isolated according to the method given under paraxanthine, on page 322. It may also be obtained from the urine by HOFMEISTER's method. The urine, acidulated with hydrochloric acid, is precipitated with phosphotungstic acid; the precipitate is decomposed by warming with baryta, filtered, and the filtrate is freed from barium by the cautious addition of sulphuric acid. The solution is then made alkaline with ammonium hydrate, any traces of phosphates that appear are filtered off, and finally it is precipitated by addition of ammoniacal silver nitrate. The precipitate which forms consists of the silver compounds of the xanthine bodies, and is purified by dissolving in hot nitric acid, as given on page 285. Xanthine has been shown to be formed at the same time with guanine, adenine, and hypoxanthine, in the decomposition of nuclein by means of dilute acids. It may, therefore, be prepared from cellular organs according to the method given under Adenine. The method of its preparation from tea-leaves is also given elsewhere.

Xanthine is a white, granular, amorphous body, and is deposited from hot aqueous solution on cooling in colorless floccules, or as a fine powder, which, under the microscope, is seen to consist of rounded granules. When occurring in calculi, it forms compact, moderately hard, yellow or brown fragments, which, on being rubbed with the finger-nail, assume a wax-like appearance. It is difficultly soluble in cold water (about 14,000 parts), alcohol, and ether; somewhat more soluble in boiling water (about 1200 parts). It is soluble in alkalies and alkali carbonates, not bicarbonate, and from these solutions it is precipitated on neutralization with acids, or by passing carbonic acid. In warm ammonia it dissolves more readily than does uric acid or guanine, and on cooling the ammonium compound recrystallizes. It acts as a weak base, and as a weak acid; with salts of the heavy metals it forms difficultly soluble or insoluble compounds. Its basic properties, however, are weaker than those of hypoxanthine or guanine.

When xanthine is evaporated with nitric acid it leaves a lemon-yellow residue (hence its name), which is not changed by ammonium hydrate—distinction from uric acid—but with potassium hydrate becomes yellowish-red, on heating purple-red. When added to a mixture of bleaching powder and sodium hydrate in a watch-glass the solution becomes covered by a dark-green scum, which changes to a brown, and soon disappears—distinction from hypoxanthine.

By means of a very interesting synthetic reaction, xanthine may be converted into theobromine, the active constituent of Theobroma cacao. Thus, the xanthine is dissolved in a sufficient quantity of sodium hydrate, necessary to form the neutral compound $C_5H_2Na_2N_4O_2$, and this product, when treated with boiling acetate of lead, yields a white precipitate of lead xanthine, $C_5H_2PbN_4O_2$. This is dried at 130°, then heated for twelve hours at 100° with methyl iodide, when the dimethyl derivative, $C_5H_2(CH_3)_2N_4O_2$, is formed. This compound is identical with the natural theobromine, and by a similar treatment is converted into trimethyl-xanthine or caffeine. The relation of xanthine to theine (caffeine) is shown in the fact that it exists together with hypoxanthine, adenine, and possibly guanine, in fresh tea-leaves. It is, therefore, clear, that by starting from guanine of guano we can produce successively xanthine, dimethyl xanthine, and trimethyl xanthine, the last two compounds being identical with the alkaloids of theobroma and of coffee.

Nascent hydrogen converts this base into hypoxanthine, but the reverse operation, the oxidation of hypoxanthine into xanthine, has been questioned of late by KOSSEL and others. On heating, a small portion volatilizes; the greater part decomposes into ammonium carbonate, cyanogen, and hydrocyanic acid. Heated to 200° with hydrochloric acid, it decomposes with the formation of ammonia, carbonic acid, formic acid, and glycocoll (E. SCHMIDT). When bromine is allowed to act on xanthine, there is formed a substitution compound, having the formula $C_5H_3BrN_4O_2$. With potassium chlorate and hydrochloric acid it yields alloxan and urea.

Xanthine is a weak base, which dissolves in acids with the formation of salts.

The hydrochloride, $C_5H_4N_4O_2.HCl$, is difficultly soluble in water, more so than the corresponding salt of hypoxanthine, from which it is deposited in glistening six-sided plates, often forming aggregations. Its solution does not precipitate platinum chloride. The nitrate forms fine yellow crystals.

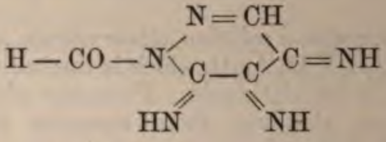
The sulphate, $C_5H_4N_4O_2.H_2SO_4 + H_2O$, crystallizes in microscopic glistening rhombic plates, decomposable by water.

With baryta water xanthine forms the difficultly soluble compound $C_5H_4N_4O_2.Ba(OH)_2$, which corresponds to the hypoxanthine salt $C_5H_4N_4O.Ba(OH)_2$, and to that of guanine.

From ammoniacal solution, silver nitrate precipitates the compound $C_5H_4N_4O_2.Ag_2O$, which is insoluble in ammonia, but soluble in hot nitric acid. From the nitric acid solution, on long standing, there separates the compound $C_5H_4N_4O_2.AgNO_3$, which, on contact with water, decomposes, giving off nitric acid. The ammoniacal solution is also precipitated by lead acetate—separation from hypoxanthine—also by calcium and zinc chlorides. Cupric acetate gives a precipitate only on boiling. The aqueous solution is not precipitated by lead acetate, but is by phosphomolybdic acid, phosphotungstic acid, by mercurous and mercuric salts. Picric acid gives an easily soluble compound, which resembles that of hypoxanthine, but differs from that of guanine.

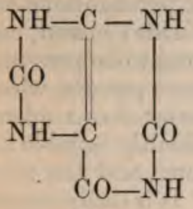
As to the physiological relation of xanthine very little need be said. It bears the same relation to guanine that hypoxanthine does to adenine, and, like the latter, is to be looked upon as an intermediate compound, a step lower than guanine, and nearer the limit of oxidation—uric acid. It is quite probable that in the body it is oxidized about as rapidly as it is formed. Like hypoxanthine, it is to be regarded as a true muscle stimulant, especially of the heart. (BAGINSKI). According to FILEHNE it produces in frogs a decided muscular rigor and paralysis of the spinal cord. The heart muscle is also affected, which is not the case with caffeine or theobromine. The fatal dose is less than one-half pro mille. In its action it is stronger than theobromine, while caffeine is weaker than either of the two. PASCHKIS and PAL hold that the reverse is true.

In closing the description of the preceding bodies it may be well to present briefly our present knowledge as to their constitution. Gautier, starting out with the idea that they are polymerization-products of hydrocyanic acid, has deduced theoretically cyclic formulæ, recalling the hexagon of the benzole derivatives. These formulæ, though formidable in appearance, are a complete failure so far as they are expressions of chemical reactions. Thus, the formula of guanine:



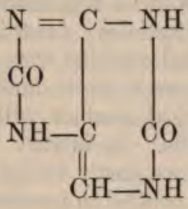
fails to show either a urea or a guanidine residue, and yet it is a well-known fact that guanine on oxidation yields parabanic acid and guanidine (page 312). In a similar manner, his xanthine formula fails to show up the urea residues which we know to be present.

Horbaczewski's synthesis of uric acid has thrown considerable light upon the constitution of these bases. As a consequence of his method of synthesis uric acid was shown to possess the structural formula given below. E. Fischer has found, as a result of experimental work, the constitution of xanthine to be expressed by the subjoined formula. We know that uric acid on treatment with nascent hydrogen is converted into xanthine, then into hypoxanthine. It follows, therefore, that a relation exists between hypoxanthine and xanthine similar to that between xanthine and uric acid. The formula of hypoxanthine, as deduced from this relation, and given below, probably represents its constitution quite closely. It is possible, however, that the CH and CO groups will be found to occupy the reverse position which they are given in this formula, in which case corresponding changes must be made in the formulæ of guanine and adenine. The latter two are based upon the relation which these bodies bear to xanthine and hypoxanthine, and cannot be said to be the result of direct experimental evidence.



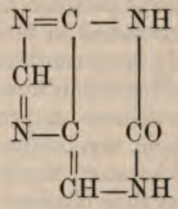
URIC ACID.

$C_5H_4N_4O_3$



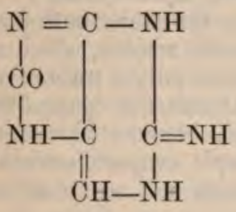
XANTHINE.

$C_5H_4N_4O_2$



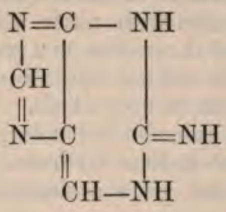
HYPOXANTHINE.

$C_5H_4N_4O$



GUANINE.

$C_5H_5N_5O$



ADENINE.

$C_5H_5N_5$.

HETEROXANTHINE, $C_6H_6N_4O_2$, is a new base which was isolated from the urine in 1884 by SALOMON. In its composition it is methyl-xanthine, and is intermediate between xanthine and paraxanthine or dimethyl-xanthine. It occurs in the urine of man and of the dog in about the same amount as paraxanthine, and the method for its isolation will be found under the description of that base. It is a remarkable fact that this base occurs in dog's urine unaccompanied by paraxanthine, and the same seems to hold true for the urine of leucocythæmic persons. SALOMON examined the liver and muscles of a dog, but was unable to obtain any heteroxanthine or paraxanthine, and the total amount of xanthine bodies present was about normal. Hence, he is inclined to think that these two bases may possibly have their origin in the kidney. Unlike the other xanthine bodies, heteroxanthine has not as yet been isolated from plants, meat extract, or guano. The amount of xanthine bodies present in the urine is unaffected by phosphorus poisoning. Neither this base nor paraxanthine has been found in bull's testicles; xanthine is also absent, and only hypoxanthine and guanine were found to be present.

Heteroxanthine forms a white amorphous powder, which sometimes on prolonged contact with water forms microscopic crystalline tufts. It is very difficultly soluble in cold water; much more easily in hot water, and the solution thus obtained is neutral in reaction. It is easily soluble in ammonium hydrate, but is insoluble in alcohol and ether. When heated it volatilizes without melting and at the same time gives off a small quantity of hydrocyanic acid. On evaporation with nitric acid on the water-bath (xanthine reaction) it remains as a pure white residue, which on contact with sodium hydrate develops only a trace of reddish coloration or none at all. WEIDEL'S test (page 328) produces a splendid red color, which becomes blue on the addition of sodium hydrate. Simple evaporation with chlorine water gives a similar though not so strong a color reaction.

Silver nitrate produces in ammoniacal, as well as in nitric acid solutions, a precipitate which readily dissolves on warming in even very dilute nitric acid; from this solution, if not too concentrated, the heteroxanthine silver nitrate compound crystallizes in well-formed plate-like prismatic crystals. Copper acetate produces in the cold, in solutions of heteroxanthine, a clear-green precipitate. It is also precipitated by phosphotungstic acid, and by ammoniacal basic lead acetate. Picric acid does not give a yellow-colored precipitate in solutions of the hydrochloride. Mercuric chloride readily precipitates heteroxanthine in the form of a grayish-yellow compound, which on standing twelve to twenty-four hours becomes converted into pure white crystalline aggregations. This mercuric compound can be converted directly into the corresponding silver compound by the addition of silver nitrate and ammonia, as described under paraxanthine.

The hydrochloride is characterized by its rather difficult solubility and ready crystallization (a distinction from the paraxanthine salt). The salt forms large colorless tufts of crystals, which on contact with water soon lose their transparency and become opaque; gradually their crystalline form disappears, till finally they completely decompose with the formation of heteroxanthine. This decomposition is hastened by warming, either with or without addition of ammonia. Platinum chloride produces in the hydrochloric acid solution a precipitate of crystalline double salt.

This base resembles paraxanthine in its property of yielding a difficultly soluble precipitate with the fixed alkali. This reaction is best brought about by dissolving the heteroxanthine hydrochloride in warm dilute sodium hydrate, when, on cooling, the corresponding sodium salt will crystallize out in oblique-angled plates. These crystals dissolve easily in water, and on neutralization of the solution with an acid a dense pulverulent precipitate of heteroxanthine forms. It can thus be distinguished from paraxanthine, the sodium compound of which, on similar treatment, yields the characteristic crystalline form of the free base. This sodium reaction, therefore, distinguishes it at once from xanthine, hypoxanthine, guanine, and paraxanthine. It differs from the latter, as has already been indicated, in the solubility and amorphous character of the free base; in the behavior of the hydrochloride and the sodium compound, and in the not giving a precipitate with picric acid, nor the characteristic odor given by paraxanthine on heating.

In its composition, heteroxanthine is, as has already been stated, methyl-xanthine and probably is related to if not identical with an isomeric body obtained synthetically by GAUTIER (see page 314). The fact nevertheless remains, that in the urine we have normally a homologous series of xanthine bodies, namely, xanthine, heteroxanthine, and paraxanthine.

PARAXANTHINE, $C_7H_8N_4O_2$, was isolated in 1883 by SALOMON, who has since shown it to be a constituent of normal urine, although present in exceedingly minute quantity. Thus from 1200 litres of urine, only 1.2 grammes (0.0001 per cent.) of this substance were obtained. It has not been found in the urine of dogs or in that of leucocythæmic patients. THUDICHUM was the first to isolate paraxanthine from the urine, and he named it urotheobromine (1879).

The method employed for the isolation of this base is, with a slight modification, that of E. SALKOWSKI, as originally proposed for the preparation of xanthine bases from urine. The urine in portions of 25 to 50 litres is made alkaline with ammonium hydrate and allowed to stand twenty-four hours. The clear supernatant fluid is decanted from the precipitate of phosphates and treated with silver nitrate (0.5 to 0.6 gramme per litre). The grayish precipitate of xanthine compounds which forms is transferred to a filter and washed with water till free from chloride; it is then suspended in water and decomposed with a current of hydrogen sulphide. The liquid is filtered by decantation and the filtrate is evaporated to dryness; the residue is extracted with 3 per cent. sulphuric acid to remove uric acid; the solution thus obtained, after it has been rendered alkaline with ammonia, is precipitated by silver nitrate.

A better procedure is to concentrate the filtrate directly over the flame or on the water-bath, till the uric acid begins to crystallize out. It is then filtered, and the filtrate, after diluting somewhat with water, is rendered alkaline with ammonium hydrate in order to precipitate any remaining uric acid and phosphates. The whole is allowed to stand one or two days, then filtered, and the filtrate again precipitated with silver nitrate. The thoroughly washed precipitate of the xanthine compounds, now free from uric acid, is dissolved in as little as possible of hot nitric acid of specific gravity 1.1, to which a little urea has been added, and the clear solution is set aside for twenty-four hours. The silver salt of hypoxanthine crystallizes from the solution and is filtered off. It can be purified by repeated recrystallization from hot nitric acid, containing a little urea, then decomposed with hydrogen sulphide, and the filtrate, rendered alkaline with ammonium hydrate, is concentrated to a small volume. On standing, pure hypoxanthine crystallizes out. The filtrate from the silver salt of hypoxanthine on being rendered alkaline with ammonium hydrate gives a precipitate which formerly was regarded as consisting entirely of the xanthine silver compound, but which from the investigations of SALOMON, has been shown to be a mixture of the salts of xanthine, paraxanthine, and heteroxanthine.

The separation of these bases is effected by the solubility of the free bases in ammonium hydrate. For this purpose the precipitate of the mixed silver salts is decomposed with hydrogen sulphide, and the filtrate, rendered ammoniacal to remove traces of phosphates and oxalates, is moderately concentrated. After standing twenty-four hours, heteroxanthine crystallizes out, partly in finely formed sheaves and tufts of needles, partly in radially striated masses. The fluid is decanted from the crust of heteroxanthine which forms in the bottom of the beaker, and after being concentrated somewhat is again allowed to stand. In this way a second crop is obtained, and this is repeated till finally the separated masses scarcely give a precipitate with sodium hydrate. All the heteroxanthine is now united and dissolved in a little hot water by the aid of sodium hydrate. After twenty-four hours the greater part of the heteroxanthine crystallizes out in bunches of crystals of sodium heteroxanthine, while a small part together with any traces of xanthine remains in solution. The crystalline mass is dried by pressure, dissolved in a little water, and the solution neutralized by addition of hydrochloric acid, when the heteroxanthine separates as a pulverulent precipitate. To remove any traces of paraxanthine, dissolve in hydrochloric acid; on standing forty-eight hours the heteroxanthine salt separates, while the easily soluble salt of paraxanthine remains in solution. To obtain the pure free heteroxanthine, the hydrochloric salt is evaporated with ammonium hydrate; the well-washed residue of heteroxanthine is then dissolved in dilute ammonia, the solution filtered, evaporated slowly, and the precipitate which forms is finally washed with alcohol and ether.

The original ammoniacal mother-liquors of heteroxanthine yield on further concentration amorphous floccules of xanthine, which are removed by filtration; from the filtrate, when concentrated still more, paraxanthine crystallizes out.

Paraxanthine is obtained in colorless, glassy, generally six-sided plates, which are arranged in tufts or rosettes. From very concentrated aqueous solutions it crystallizes in long, colorless, interwoven needles, which on drying exhibit the silky lustre of tyrosin. The crystals belong to the monoclinic system, and may crystallize with as well as without water. If water is present on careful heating (110°) the crystals lose their brilliancy and become whitish and opaque, and at 120°–130° the water is completely driven off. The conditions under which crystals containing water are formed are not known; probably by slow crystallization, whereas rapid crystallization from hot concentrated solution yields the anhydrous needles. At about 170°–180° sublimation takes place. The melting-point is at about 284° (KOSSEL). It can be heated to 250° without melting or suffering any decomposition, but when heated more strongly it gives off white vapors which possess a distinct iso-nitril odor, at the same time it carbonizes and takes fire. When evaporated with concentrated nitric acid, as in the ordinary xanthine test, it gives only a slight yellow residue. On the other hand, WEIDEL's test, evaporation with chlorine water containing a trace of nitric acid, and then placing the dry residue into an ammoniacal atmosphere under a bell-jar, gives a beautiful rose-red color.

It is difficultly soluble in cold water (though more easily than xanthine); somewhat more readily soluble in hot water, and insoluble in ether and alcohol. It is soluble in ammonium hydrate, hydrochloric acid, and nitric acid. Its solutions are neutral in reaction.

Silver nitrate produces in nitric acid, as well as in ammoniacal solutions, a flocculent or gelatinous precipitate, which in concentrated solutions forms an almost perfect jelly-like mass. This silver precipitate is soluble in warm nitric acid, from which on cooling it separates in white crystalline tufts possessing a silky lustre. On decomposition with hydrogen sulphide the silver salt yields pure paraxanthine. Picric acid produces in the hydrochloric acid solution a precipitate consisting of densely felted yellow crystalline spangles.

It is also precipitated by phosphotungstic acid and copper acetate; mercuric chloride when added in excess gives a precipitate composed of a mass of colorless prisms, which are rather difficultly soluble in cold water; easily in hot water. The crystals of paraxanthine mercuric chloride when moderately heated become opaque from loss of water of crystallization; at a higher temperature they melt, undergoing at the same time partial decomposition, and on strong heating they evolve disagreeable nauseating vapors. The aqueous solution of this mercuric double salt gives with silver nitrate an abundant precipitate of silver chloride, which disappears on the addition of ammonium hydrate and is replaced by the flocculent gelatinous precipitate of silver paraxanthine. The hydrochloric acid solution of paraxanthine crystallizes with difficulty even when strongly concentrated, and on the addition of platinum chloride it yields a well-crystallizable orange-colored paraxanthine platinochloride. It is not precipitated by basic lead acetate nor by mercuric nitrate.

In its behavior to the xanthine test this base resembles hypoxanthine, whereas in giving WEIDEL's reaction it approaches xanthine. Finally, it coincides with guanine by yielding a precipitate with picric acid. Although it thus agrees in some of its reactions with all three of these xanthine bodies, it can, however, be easily distinguished from them by its behavior with the fixed alkalies. Sodium or potassium hydrate dissolves these bases and holds them in solution, but when added to concentrated paraxanthine solution the alkali produces a precipitate of long, glittering, crystalline spangles, which under the microscope are seen to consist of delicate rectangular, often longitudinally striated, plates which are either isolated or united in tufts. Besides these crystals there are also present hexagonal plates resembling cystin. The crystals are soluble in a little water, or on warming, but precipitate again on cooling. Paraxanthine, however, shares with heteroxanthine the property of forming a difficultly soluble compound with the fixed alkalies, but can be distinguished from the latter by neutralizing with an acid the solution of the sodium or potassium compound, when, in the case of paraxanthine, there will be obtained a precipitate of the characteristic crystals of that base; whereas heteroxanthine is obtained on similar treatment as a dense pulverulent precipitate. This reaction is not given by theophylline.

It is interesting to observe that paraxanthine is isomeric with theobromine, theophylline, and also with a body recently described by FISCHER as dioxy-dimethyl-purpurine. In its composition it is, therefore, a dimethyl-xanthine.

The physiological action of paraxanthine has been studied by SALOMON. Injections into the muscles of 1–2 mg. produced almost at once a *rigor-mortis*-like condition of the muscles affected, with diminished reflex excitability without previous increase; 6–8 mg. introduced into the lymph sac brings on a gradual loss of voluntary motion as well as of reflex excitability; the rigor is more marked in the anterior extremities, which have a wooden or waxy consistency. Dyspnœa is likewise an early symptom, but as soon as rigor sets in the respirations drop far below the normal, and may even be absent for several minutes. At times the lungs are enormously dilated, same as in theobromine. The heart's action is intact till the very last. In mice the reflexes are increased almost to a tetanus. The lethal dose for frogs, subcutaneously, was found to be 0.15–0.2 per cent. of the body-weight—somewhat lower than that of theobromine and xanthine. The action of these three bases is very similar. They produce in common the slow creeping movements, followed by cessation of spontaneous muscle action, complete loss of reflex excitability without a previous rise, and the heart's action is not affected till in the latest stages.

CARNINE, $C_7H_8N_4O_3$, was isolated in 1871 from American meat-extract by WEIDEL, but has not been obtained from muscle-tissue itself. It has also been obtained from yeast liquors by **Schützenberger**, and from urine by **Pouchet**. It can be separated from the meat-extract, of which it forms about one per cent., by the following method originally employed by **Weidel**. The extract is dissolved in six or seven parts of warm water, then concentrated baryta water is added, avoiding, however, an excess. The filtrate is precipitated by basic lead acetate. The precipitate is collected, thoroughly washed and pressed, and finally it is repeatedly extracted with a large quantity of boiling water. The carnine lead salt is thus dissolved out; the filtrate, after removal of the lead by hydrogen sulphide, is evaporated to a small volume. The concentrated solution thus obtained is treated with silver nitrate, which gives a precipitate of silver chloride and of the silver salt of carnine. By treatment with ammonium hydrate the silver chloride can be completely removed from the precipitate, whereas the silver compound of carnine is insoluble in that reagent. To obtain pure carnine the silver salt is decomposed with hydrogen sulphide, and the filtrate, after purification by bone-black, is evaporated to crystallization.

Carnine forms white crystalline masses, which on drying become loose and chalk-like. It is very difficultly soluble in cold water, easily and completely in boiling water, and recrystallizes on cooling. It is insoluble in alcohol and ether. The taste is decidedly bitter, and the reaction is neutral. The base is not precipitated by neutral lead acetate, but is precipitated by the basic salt as a flocculent white precipitate, soluble in boiling water. On heating, carnine decomposes and takes fire, and at the same time gives off a peculiar odor. It crystallizes with one molecule of water, which it loses at 100°–110°.

The hydrochloride, $C_7H_8N_4O_3.HCl$, is crystalline, and decomposes on heating with concentrated hydrochloric acid.

The platinochloride, $C_7H_8N_4O_3.HCl.PtCl_4$, forms a fine, sandy, golden-yellow powder.

With silver nitrate, carnine unites to form a white flocculent precipitate, insoluble in nitric acid or in ammonium hydrate. Its formula corresponds to $2(C_7H_7AgN_4O_3)+AgNO_3$.

Carnine is not affected by prolonged boiling with concentrated barium hydrate. Bromine water decomposes it with the evolution of gas and the formation of hypoxanthine. This change takes place according to the following equation:

$$C_7H_8N_4O_3 + 2Br = C_5H_4N_4O.HBr + CH_3Br + CO_2.$$

A similar decomposition into hypoxanthine is brought about by the action of nitric acid, though in this case oxalic acid and a yellow body are formed. When carnine is evaporated with chlorine water containing a little nitric acid, the residue, on contact with ammonia, gives a rose-red color (murexide test). This is due, according to WEIDEL, to the formation of hypoxanthine, but it has since been shown that the latter base does not give this reaction, and hence it is due to the production of xanthine, or some similar body.

The physiological action of carnine has been examined somewhat by BRÜCKE, and according to him it is not very poisonous. The only effect observed, when taken internally, was a fluctuation in the rate of the heart-beat, though even this was by no means definite in its nature.

A BASE, $C_4H_5N_5O$, was obtained by GAUTIER from fresh muscle tissue of beef, according to the method given on page 334, and on account of a resemblance in some of its properties with xanthine, he named it pseudoxanthine. This name is very inappropriate, not only because it differs so much in its empirical formula from that of xanthine, $C_5H_4N_4O_2$, but also because the term pseudoxanthine has already been applied by SCHULTZEN and FILEHNE to a body isomeric with xanthine, which was obtained by the action of sulphuric acid on uric acid.

The free base forms a light-yellow powder, slightly soluble in cold water, soluble in weak alkali and in hydrochloric acid. The hydrochloride is very soluble, and it forms stellate prisms with curved faces, which resemble the corresponding salt of hypoxanthine, and to some extent, also, the whetstone-shaped crystals of uric acid.

Like xanthine, its aqueous solution is precipitated in the cold by mercuric chloride, silver nitrate, and by ammoniacal lead acetate, but not by normal lead acetate. On evaporation with nitric acid, the residue gives, on contact with potassium hydrate, as in the case of xanthine, a beautiful orange-red coloration (xanthine reaction). It differs from xanthine, not only in its empirical composition, but also in its greater solubility, and in its crystalline form. It is possible that this base, on account of its great resemblance to xanthine, may have been mistaken, at different times, for that compound.

GERONTINE, $C_5H_{14}N_2$, is a new base which was isolated by GRANDIS in 1890. It has been repeatedly observed in the form of peculiar crystals found in the cell nuclei in the liver, particularly of old dogs. The free base is an isomer of cadaverine, etc., and resembles it somewhat. It crystallizes in needles which are readily soluble in water and alcohol; possesses a strongly alkaline reaction, and yields the ordinary alkaloidal reactions.

The hydrochloride forms prismatic crystals, which are deliquescent and easily soluble in alcohol.

The platinochloride, $C_5H_{14}N_2.2HCl.PtCl_4$, is soluble in water and crystallizes in spindle-shaped needles, arranged in rosettes. It decomposes at 115°.

The gold salt forms small needles, and is easily soluble in water and alcohol.

It combines with one molecule of mercuric chloride to form deliquescent cubes or rectangular prisms containing two molecules of water of crystallization. It decomposes above 100°. This distinguishes it from cadaverine, which combines with three to four molecules of mercuric chloride. The crystals observed in the liver are probably the phosphate.

The new base also yields a benzoyl compound which melts at 175°–176°.

Physiological Action.—It seems to exert a paralyzing action upon the nerve centres, and leaves the nerves and muscles unaffected.

SPERMINE, C_2H_5N, or $C_{10}H_{26}N_4$ (?), is the basic substance obtained by SCHREINER (1878) from semen, calf's heart, calf's liver, bull's testicles, from the organs of leucocythæmics, and also from the surface of anatomical specimens kept under alcohol. In 1888 KUNZ reported the presence of a non-poisonous base, C_2H_5N, spermine or ethyleneimide in cholera cultures. In this case it occurs, then, as a ptomaïne. A confirmation of the identity of the two bases is necessary. Previous to this, however, it had been known for a long time under the name of "CHARCOT-NEUMANN or LEYDEN crystals," which are the phosphate of spermine. These peculiarly shaped crystals have been found in the sputa of a case of emphysema with catarrh, in the bronchial discharges in acute bronchitis, as well as in sputa of chronic bronchitis, in the blood, spleen, etc., of leucocythæmics and anæmics, and in the normal marrow of human bones, as well as in human semen. Altogether it seems to have a very wide distribution, especially in certain diseases, as in leucocythæmia.

It can be prepared from fresh human semen in the following manner: The semen is washed out of linen by a little warm water, evaporated to dryness, boiled with alcohol, and the insoluble portion is allowed to subside by standing some hours. The precipitate is filtered off, washed, and dried at 100°. This residue, containing the spermine phosphate, is triturated, and then extracted with warm ammoniacal water. From this solution, on slow evaporation, the phosphate crystallizes in its peculiar-shaped crystals.

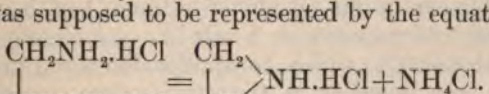
The free base is obtained, on decomposing the phosphate with baryta and evaporating the filtrate, as a colorless liquid, which, on cooling, crystallizes. From alcohol it crystallizes in wavellite-shaped crystals, which readily absorb water and carbonic acid from the atmosphere. They are readily soluble in water and in absolute alcohol, almost insoluble in ether, and possess a strongly alkaline reaction. When heated with platinum it gives off thick, white fumes, and a weak ammoniacal odor. With potassium bismuth iodide it yields orange-colored crystalline floccules, which, under the microscope, appear as long, sharp, plumose needles—distinction from diethylenediamine. The aqueous solution of the base is precipitated by phosphomolybdic and phosphotungstic acids, tannic acid, gold and platinum chlorides. It cannot be volatilized from alkaline solution by steam without undergoing decomposition (Majert and Schmidt). It is not poisonous.

The hydrochloride, $C_2H_5N.HCl$ (?), crystallizes in six-sided prisms, united in tufts, and is extremely soluble in water, almost insoluble in absolute alcohol and ether.

The aurochloride, $C_2H_5N.HCl.AuCl_3$ (?), forms shining, golden-yellow, irregular plates, and when freshly precipitated it is easily soluble in water, alcohol, and ether, but the dried salt is incompletely soluble in water. The aqueous solution, treated with magnesium, gives off a sperm-like odor. The platinochloride crystallizes in prisms.

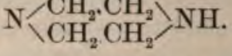
The phosphate, $(C_2H_5N)_2.H_3PO_4+3H_2O$ (?), forms prisms and slender double pyramids arranged in rosettes. It is difficultly soluble in hot water, insoluble in alcohol, easily soluble in dilute acids, alkalies, and alkali carbonates. It melts with decomposition at about 170°. It is probable that the above formula does not represent the salt as found, and from theoretical considerations Ladenburg was inclined to think that Schreiner's phosphate had the composition $(C_2H_5NH)_4Ca(PO_4)_2$.

Ladenburg and Abel prepared in 1888 a compound, ethyleneimine, which was first supposed to be isomeric with spermine. The reaction whereby it is prepared is similar to the one by which Ladenburg effected the synthesis of piperidine. Ethylenediamine hydrochloride is subjected to dry distillation, when it decomposes into ammonium chloride and the hydrochloride of the new base. The reaction was supposed to be represented by the equation:



$$\begin{array}{l} CH_2NH_2.HCl \\ | \\ CH_2NH_2.HCl \end{array} = \begin{array}{l} CH_2 \\ | \\ CH_2 \end{array} \!\!> NH.HCl + NH_4Cl.$$

Since then Ladenburg has shown that the boiling-point of this compound did not agree with what it should be theoretically, if represented by the above formula. A determination of the vapor density showed that the molecular weight was twice that corresponding to the formula given, and hence was $C_4H_{10}N_2$. Majert and Schmidt assuming spermine to be ethyleneimine, as was apparently shown by Ladenburg and Abel's investigation, attempted to prepare the latter on a manufacturing scale with the expectation that it might be used as a substitute for Brown-Séquard's testicular fluid. They were soon able to show, however, that ethyleneimine did not possess the composition assigned to it, but that it was identical with Hofmann's diethylenediamine (piperazine),



$$N\begin{matrix}\diagup CH_2.CH_2 \diagdown \\ \diagdown CH_2.CH_2 \diagup\end{matrix}NH.$$

This was soon confirmed by Hofmann and by Ladenburg. Spermine was then assumed to be identical with piperazine, but recently (1891) Majert and Schmidt compared some spermine from Schreiner with their own piperazine and found the two bases to be distinct, especially with reference to the phosphates and the potassium bismuth iodide precipitates.

About the same time (1891) Poehl announced that the composition of spermine was more complex than what it had been hitherto supposed to be. He ascribed to it the formula $C_{10}H_{26}N_4$. The formula of the platinum salt corresponded to $C_{10}H_{26}N_4.4HCl.2PtCl_4$; and that of the gold salt was represented by $C_{10}H_{26}N_4.4HCl.4AuCl_3$.

From this it would appear that spermine is essentially distinct from piperazine. The composition and structure of this interesting base must therefore be considered as not settled.

The nuclein of the spawn of salmon has been found by Miescher to exist in a salt-like combination with a basic substance, to which he applied the name protamine. Picard has found it in the same source, together with hypoxanthine and guanine, but no xanthine. The formula assigned to this base is quite complex, and cannot be considered as definitely settled. Analysis of the platinochloride gave: Pt=24.64, Cl=26.45, N=15.03, C=22.80, H=4.15, O=6.93. The hydrochloride forms an amorphous, hygroscopic, sticky mass.

LEUCOMAÏNES OF THE CREATININE GROUP.

The knowledge of the formation of basic substances (ptomaïnes) during the putrefaction of nitrogenous organic matter, led to a series of investigations having for their object the isolation of alkaloidal bodies, if such existed, from the normal living tissues of the organism. A number of compounds possessing alkaloidal properties, such as the xanthine derivatives, already described, had been known for a long time, although their physiological relation to the animal economy was little, if at all, understood. GUARESCHI and MOSSO, in the course of their researches on ptomaïnes, were among the first to direct their attention to the possible presence of ptomaïne-like bodies in fresh tissues. They obtained in those cases where the extraction was carried on without the use of acids, only very minute traces of an alkaloidal body (possibly choline), and an inert substance, methyl-hydantoïn, which, although it can scarcely be classed as a basic compound, is closely related to creatine, and for this reason will be described at the end of this section. Other Italian chemists, as PATERNÒ and SPICA and MARINO-ZUCO, had also shown that the normal fluids and tissues of the body were capable of yielding substances alkaloidal in nature, and these were regarded by them as identical with, or similar to, the ptomaïnes of SELMI.

Arginine, $C_6H_{14}N_4O_2$, is a base obtained by SCHULZE from the conglutin of lupine sprouts, and according to him it is related to creatinine and possibly to the leucomaïnes of GAUTIER. Lysatine, $C_6H_{13}N_3O_2$, and lysatinine, $C_6H_{11}N_3O$, are analogous bases, obtained by DRECHSEL from casein (page 242). These three bases can properly be looked upon as important sources of the nitrogenous bases found in animals and plants.

LIEBREICH, in 1869, discovered in normal urine an oxidation-product of choline, probably identical with betaine (pp. 249 and 343), and **Pouchet**, in 1880, announced the presence in the same secretion of allantoin, carnine (page 344), and an alkaloidal base, which, however, was not obtained at that time in sufficient quantity to permit a determination of its character. Subsequently he succeeded in isolating this base as well as another closely related body, both of which will be described in their proper place. **Gautier** has been engaged for a number of years in the study of the leucomaïnes occurring in fresh muscle tissue, and he has succeeded in isolating several new compounds.

A number of these substances are credited with possessing an intensely poisonous action, and if such is the case it is very evident that any undue accumulation of such bases in the system, resulting from an interference in the elimination, may give rise to serious disturbances. The amount of these substances present in the daily yield of the urine is very small—so small, indeed, that we must rather look upon this small quantity as having escaped oxidation in the body. It is well known that the living tissues possess an enormous oxidizing and reducing power, and, according to **Gautier**, there is constantly going on in the normal tissues of the body a cycle of changes—the formation of leucomaïnes and their subsequent destruction by oxidation, before they have accumulated in sufficient quantity to produce poisonous effects.

The following method was employed by **Gautier** in his study of the leucomaïnes of muscle tissue: The finely divided fresh beef-meat or the Liebig's meat extract is treated with twice its weight of water, containing 0.25 gramme of oxalic acid, and one to two c.c. of commercial peroxide of hydrogen per litre. The purpose of these precautions is to prevent fermentation. At the end of twenty-four hours the liquid is raised to the boiling-point, then filtered through linen, and the residue is thoroughly squeezed. The filtrate is again raised to the boiling-point in order to coagulate any remaining albumin, and finally filtered through paper. The clear liquid thus obtained is evaporated in a vacuum at a temperature not exceeding

50°, and the acid syrupy residue is extracted with 99 per cent. alcohol; the alcoholic extract is in turn evaporated in a vacuum, and the residue taken up with warm alcohol of the same strength. The filtered alcoholic solution is set aside for twenty-four hours, and any deposit which forms is removed by filtration; ether (65°) is then added as long as a precipitate continues to form, and the whole is again allowed to stand for twenty-four hours. The ether-alcoholic filtrate from this precipitate is evaporated first on the water bath, and finally in a vacuum; the slight residue obtained contains a small quantity of basic substances possessing an odor of hawthorn.

The syrupy precipitate produced by the ether partially crystallizes on standing; a little absolute ether is then added, and after standing several days more the liquid is separated by means of an aspirator from the deposit of crystals (A). These are first washed with 99 per cent. alcohol, and then extracted with boiling 95 per cent. alcohol. The alcoholic solution, concentrated by evaporation, gives, on cooling, a deposit of lemon-yellow-colored crystals of xantho-creatinine (B), from the mother-liquor of which there separates a crop of new crystals (C). The residue of the crystals (A) left after treatment with the boiling 95 per cent. alcohol is extracted with boiling water, which afterward gives a slight deposit of yellowish-white crystals of amphi-creatine (D). The aqueous mother-liquors on concentration yield another deposit of orange-colored crystals of cruso-creatinine (E). GAUTIER has, furthermore, separated three other bases from the mother-liquors of the crystals obtained as above. Thus, a base which he named pseudoxanthine is stated to have been obtained by evaporating the alcoholic mother-liquors of B, D, E (?) in a vacuum, taking up the residue with water, and precipitating the hot solution with copper acetate. The precipitate is decomposed with hydrogen sulphide, and the aqueous solution, filtered while boiling-hot, yields a deposit of a sulphur-yellow powder of pseudoxanthine. Thus, by the use of alcohol, ether, and water, GAUTIER, according to his statement, has succeeded in obtaining a sharp separation between these bases. The importance of the subject is such as to require not only confirmation of the results arrived at by GAUTIER, but also a more detailed and exact study of the chemical and physiological behavior of these bodies.

To the physiological chemist these substances are of especial interest because of the possible relation which they bear to the formation of creatine and creatinine in the muscle. It will be seen that in the leucomaïnes of this group, as well as in those of the uric acid group, hydrocyanic acid plays a very important part in the molecular structure of these bases. Just what the function of this cyanogen group is, so far as the vital activity of the tissues is concerned, we know very little, though recent investigations seem to show that the seat of the cyanogen formation lies within the nucleated cell, and is intimately connected with the functions of the nuclein molecule.

CRUSO-CREATININE, $C_5H_8N_4O$, forms orange-yellow crystals which are slightly alkaline in reaction, and possess a somewhat bitter taste. It yields a soluble, non-deliquescent hydrochloride crystallizing in bundles of needles; also a soluble platinochloride which forms tufts of beautiful, slender prisms. The aurochloride is obtained as slightly soluble, crystalline grains, and, like the platinum double salt, is partially decomposed on heating. It is not precipitated by acetate of zinc or by mercuric nitrate, but is precipitated in the cold by solutions of alum. Zinc chloride produces in somewhat concentrated solutions a pulverulent precipitate which dissolves on heating, and recrystallizes again when it cools. Like xantho-creatinine, it is not thrown out of solution by oxalic or nitric acid, and is thus distinguished from urea and guanidine; nor is it precipitated by acetate of copper—a distinction from xanthine derivatives. Mercuric chloride produces an abundant flocculent precipitate which on heating partially dissolves, decomposing at the same time. Sodium phosphomolybdate gives a heavy yellow precipitate, whereas potassium mercuro-chloride and iodine in potassium iodide have no effect. Potassium ferricyanide is not reduced. This base differs in its composition from creatinine by HCN, the elements of hydrocyanic acid, but in its crystalline form and alkaline reaction, and some other properties, it would seem to be closely related to this latter substance. Because of this apparent relationship and its golden-yellow color, GAUTIER named it cruso-creatinine.

XANTHO-CREATININE, $C_5H_{10}N_4O$, is the most abundant of muscle leucomaïnes. It crystallizes in sulphur-yellow, thin spangles, consisting of nearly rectangular plates which resemble somewhat those of cholesterin. It is soft and talc-like to the touch; possesses a slightly bitter taste, and when dissolved in boiling alcohol it gives off the odor of acetamide, though ordinarily in the cold it has a slight cadaveric odor. When heated, the substance evolves an odor of roast meat, carbonizes in part, and yields ammonia and methylamine. The crystals are amphoteric in reaction, are soluble in cold water, and can be recrystallized from boiling 99 per cent. alcohol.

It forms a hydrochloride crystallizing in plumose needles, and a very soluble platinochloride; the aurochloride crystallizes with difficulty. Like creatinine, it is precipitated by zinc chloride; the yellowish-white precipitate dissolves with partial dissociation on warming, and on cooling separates as isolated or stellate groups of fine needles which possess the composition $(C_5H_{10}N_4O)_2ZnCl_2$. Silver nitrate throws down, in the cold, a flocculent precipitate which likewise dissolves on heating, and recrystallizes in needles. Mercuric chloride produces a yellowish-white precipitate. It is not precipitated by oxalic or nitric acid, nor by potassio-mercuric chloride, or iodine in potassium iodide. Tannin produces in time a slight turbidity, while sodium phosphomolybdate gives a heavy yellowish precipitate. This base is distinguished from the members of the uric acid group by not giving a precipitate with copper acetate, not even on heating.

On gentle oxidation with potassium permanganate it is converted into a black substance insoluble in acids and alkalies, and resembling azulmic acid. By treatment with recently precipitated mercuric oxide, it yields a substance which can be recrystallized from boiling 93 per cent. alcohol in needles which possess a slight alkaline reaction, and forms a slightly soluble, crystalline platinochloride. This new substance is precipitated from alcoholic solution by the addition of ether, as a mass of beautiful, white, silky needles resembling caffeine. These crystals melt at 174°; caffeine melts at 178°.

Xantho-creatine, given in fairly large doses, is poisonous, producing in animals depression, somnolence, and extreme fatigue, accompanied by frequent defecation and vomiting. In its general properties this base resembles creatinine very much, and it was on account of this resemblance and its yellow color that it was named xantho-creatinine. This relation becomes especially evident since this base appears in the physiologically active muscle at the same time with creatinine, constituting sometimes one-tenth of the creatinine present. MONARI has found this base in the aqueous extract of the muscles of an exhausted dog, and also in the urine of soldiers tired by several hours' march. He also demonstrated its presence in the urine of a dog after previous injection of creatinine.

AMPHI-CREATINE, $C_9H_{19}N_7O_4$, is slightly soluble and crystallizes from boiling water in yellowish-white oblique prisms, which possess, if any, a slightly bitter taste. When heated to 100° it decrepitates somewhat, and at 110° it becomes opaque white. Potassium hydrate does not decompose it in the cold. Although a weak base, it combines to form salts just as the preceding members of this group. The hydrochloride is crystalline, and is not deliquescent; the platinochloride forms rhombic plates, which are soluble in water, but are insoluble in absolute alcohol; the aurochloride crystallizes in easily soluble, very small, microscopic crystals, which are tetrahedral to hexahedral in their habit. It is not precipitated by copper acetate or by mercuric chloride; nor does it give the murexide test, or the xanthine reaction. Sodium phosphomolybdate produces a yellow, pulverulent precipitate. In its properties it resembles creatine, and indeed GAUTIER thinks it may be possibly a combination of creatine, $C_4H_9N_3O_2$, and a base $C_5H_{10}N_4O_2$, which, it will be seen, differs from the former only by a HCN group. This second compound, if it really exists, has an analogy in cruso-creatinine, the relation of which to creatinine may be expressed by the equation:

$$\underset{\text{Cruso-creatinine.}}{C_5H_8N_4O} = \underset{\text{Creatinine.}}{C_4H_7N_3O} + HCN.$$

In a similar manner, amphi-creatine may be regarded as

$$\underset{\text{Amphi-creatine.}}{C_9H_{19}N_7O_4} = \underset{\text{Creatine.}}{2C_4H_9N_3O_2} + HCN.$$

A Base, $C_{11}H_{24}N_{10}O_5$, was isolated by Gautier from the mother-liquors of xantho-creatinine. It crystallizes in colorless or yellowish, thin, apparently rectangular plates, which are tasteless, and possess an amphoteric reaction. The hydrochloride forms bundles of fine needles; the sulphate yields a confused mass of needles; the platinochloride is soluble, non-deliquescent, and crystalline. When heated with water in a sealed tube at 180°–200°, it gives off ammonia and carbonic acid, and is converted into a new base, which, however, has not been studied. This reaction may be expressed by the equation:

$$C_{11}H_{24}N_{10}O_5 = 2C_5H_{10}N_4O_2 + \underset{\text{Urea.}}{CO(NH_2)_2}.$$

The urea which at first forms, is, in turn, decomposed, thus:

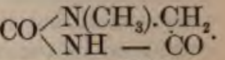
$$CO(NH_2)_2 + H_2O = CO_2 + 2NH_3.$$

It is to be observed that this base differs in composition from the following one by HCN, the hydrocyanic acid molecule.

A Base, $C_{12}H_{25}N_{11}O_5$, was obtained from the mother-liquors of cruso-creatinine, and forms rectangular silky plates, resembling those of the preceding base and of xantho-creatinine. It forms crystallizable salts.

These complex bases will require further study in order to elucidate their physiology, and the possible connection which they may have with the formation of urea, and of the creatinine derivatives already described.

Methyl-hydantoïn, $C_4H_6N_2O_2$, =



$CO\left\langle\begin{matrix}N(CH_3).CH_2\\ NH\ -\ CO\end{matrix}\right.$.

—This substance was obtained by Guareschi and Mosso (1883), by extracting fresh meat with 1–1.5 volumes of water (without addition of acid), for two hours at 50°–60°. The aqueous extract was evaporated on the water-bath and the residue was extracted with 95 per cent. alcohol. This alcoholic solution, after the alcohol was driven off, was taken up in water, filtered, and the aqueous solution was first extracted with ether, then rendered alkaline with ammonia, and again extracted with ether. The alkaline ether extract gave on evaporation a white crystalline residue of methyl-hydantoïn. The amount of this substance present in flesh appears to be quite variable, since, at times, none whatever can be extracted. Albertoni has isolated it from dog's flesh. Previous to its discovery in flesh by Guareschi and Mosso, it was known for a long time as a decomposition-product of various nitrogenous bases of the body. Thus, Neubauer prepared it by heating creatinine with barium hydrate, while Huppert obtained it by fusing together sarcosine with urea. As it occurs in muscle it is probably derived from the creatine, though under what conditions this splitting up takes place is not definitely known. Acetic and lactic acids are incapable of effecting this change. At all events, it belongs to the ureides, and is intermediate between creatinine, sarcosine, and urea Compare the above formula with that of creatinine, p. 226.

Methyl-hydantoïn forms prisms which are easily soluble in water and alcohol, and but slightly soluble in cold ether. It melts at 156° (Salkowski); at 159°–160° (Guareschi and Mosso). Its aqueous solution is slightly acid in reaction. On strong heating it volatilizes. When fused with potassium hydrate it gives off ammonia; it reduces mercuric nitrate in the cold. Treated with mercuric oxide it assumes an alkaline reaction, and the filtrate on heating yields metallic mercury. With silver oxide it forms pearly lanceolate plates having the composition $C_4H_5N_2O_2.Ag$. It does not give any alkaloidal reactions.

UNDETERMINED LEUCOMAÏNES.

Leucomaïnes of Expired Air.

It was shown at quite an early period that exhalations from animals contain, besides an increased amount of carbonic acid, some organic matter, the nature of which, on account of the exceedingly minute quantity in which it occurs, has never been satisfactorily determined. Nevertheless, various observers did not hesitate to ascribe to it the ill effects consequent upon breathing impure air, while at the same time the carbonic acid formed during respiration was considered as either entirely inert or as insignificant in its action. Thus, respired air from which moisture and carbonic acid have been removed, but which still contains the organic vapors, has been found to be highly poisonous. On the other hand, if the respired air is drawn through a red-hot tube to destroy the organic matter, the air thus purified is capable of sustaining life even in presence of a large percentage of carbonic acid. While it cannot be, therefore, doubted that the organic matter of expired air plays a most important part in producing the well-known noxious effects resulting from breathing confined and vitiated air, nevertheless it would seem from experiments made by ANGUS SMITH that the increase of even such small quantities of carbonic acid in the air, as from 0.04, the normal amount present, to 0.1 per cent., is capable of producing systemic disturbances characterized by a decrease in the pulse-rate and an increase in the rate of respiration.

SMITH is consequently of the opinion that the constant lowering of the pulse in impure air occasioned by the presence of carbonic acid must have a depressing effect on the vitality. Whatever ill effects the carbonic acid may produce of itself, it remains certain that this gas is not the most potent and most injurious constituent of respired air;

and the investigations of HAMMOND, NOWAK, SEEGEN, and others, point conclusively to the organic matter as the direct and immediate agent which produces those symptoms of sickness and nausea experienced in badly ventilated closed rooms.

Of special importance to the sanitarian and physician is the work on the nature and action of the poisonous principle of expired air which has been done by BROWN-SÉQUARD, D'ARSONVAL, and R. WURTZ. The first two observers found that the vapors exhaled by dogs, when condensed, and the aqueous liquid (20–44 c. c.) thus obtained was injected into other animals, death was produced, generally within twenty-four hours. The symptoms observed were dilatation of the pupil, increase of heart-beat to 240–280 per minute, which may last for several days or even weeks, while the temperature remains normal; the respiratory movements are generally slowed, and usually there is observed paralysis of the posterior members. Choleraic diarrhœa is invariably present. As a rule, it appears that larger doses cause labored respiration, violent retching, and contraction of the pupil. A rapid lowering of temperature, 0.5° to 5°, is sometimes observed. These same symptoms, apparently in aggravated form, were obtained when the liquid had been previously boiled for the purpose of destroying any germs that might be present. The appearances presented on post-mortem were much like those observable in cardiac syncope.

The above work has been confirmed, in part, by R. WURTZ, who, by passing expired air through a solution of oxalic acid, has obtained besides ammonia a volatile organic base which is precipitated by BOUCHARDAT'S reagent and by potassio-mercuric iodide. It is said to form a platinum double salt crystallizing in short needles, and a soluble gold salt. When heated to 100° it gives off a peculiar odor. This basic substance may properly be looked upon as a leucomaïne.

DASTRE and LOYE and LEHMANN and JESSEN have repeated the above experiments with wholly negative results. It is possible that the most highly poisonous substances formed in the body when there is an insufficient air-supply are not eliminated in the exhaled air.

Sewer-air, according to observations made by ODLING, contains a basic substance which is probably in composition a compound ammonia. It contains, however, more carbon than methylamine and less than ethylamine.

It should be remarked that JACKSON has (Dec. 1887) announced the presence in expired air of quantities of carbon monoxide gas sufficient to produce the ill effects ordinarily attributed to the organic matter. The presence of this poisonous gas must first be fully demonstrated before it can be taken into account in the consideration of the toxicity of air; certainly, even if present, it cannot explain the results obtained by the French investigators as stated above.

According to ILOSVA, expired air contains nitrous acid. This may possibly be derived from that which is constantly being formed in the mouth, probably by the reduction of nitrates (MILLER).

Leucomaïnes of the Urine.

A number of basic substances have been isolated at different times from the urine, and on that account they may be properly classed as leucomaïnes. Thus, LIEBREICH (1869) found in the urine a base which apparently was an oxidation-product of choline, and which has since been regarded as identical with betaine. In 1866 DUPRÉ and BENCE JONES found, among other things in the urine, an alkaloidal body which in sulphuric acid solution possessed a blue fluorescence (see p. 347). Most of the members of the uric acid group of leucomaïnes have been detected in the urine and on account of their well-defined nature they are described by themselves. In the urine and feces of cystinuria UDRÁNSZKY and BAUMANN discovered the well-known ptomaïnes, cadaverine and putrescine. For isolation, see pp. 207 and 208.

In 1879, THUDICHUM announced the presence in the urine of four new alkaloids, one of which, urotheobromine, was subsequently rediscovered by **Salomon** and named paraxanthine (page 321). Another base which was obtained, namely, reducine, yielded a barium salt which readily reduced the salts of silver and mercury. Its formula probably corresponds to $C_{12}H_{24}N_6O_9$ or $C_6H_{11}N_3O_4$. A third alkaloid, parareducine, formed a zinc compound having the composition $C_6H_9N_3O.ZnO$. A fourth base is said to give a compound with platinum chloride and to contain an aromatic nucleus (aromine). Besides these four bases **Thudichum** describes two other substances which he considers basic. These are urochrome, the normal pigment of the urine, and creatinine.

In 1880, **Pouchet** announced the presence of carnine, $C_7H_8N_4O_3$, and of another base which he subsequently analyzed and found to have either the composition $C_7H_{12}N_4O_2$ or $C_7H_{14}N_4O_2$. This substance formed deliquescent fusiform crystals, sometimes crystallized in bundles or irregular spheres, which possessed a slightly alkaline reaction and combined with acids to form crystallizable salts. It was soluble in dilute alcohol, almost insoluble in strong alcohol, insoluble in ether. The hydrochloride yielded double salts with gold chloride, platinum chloride, and mercuric chloride. The platinochloride formed deliquescent golden-yellow rhombic prisms. This base occurred in the dialysate (see page 265). From the non-dialyzable portion, **Pouchet** obtained another base corresponding to the formula $C_3H_5NO_2$, which he calls the "extractive matter of urine." It yields precipitates with the general alkaloidal reagents, is non-crystallizable and is altered on exposure to air and resinified by hydrochloric acid. On the addition of platinum chloride it is rapidly oxidized, but does not yield a platinochloride. The same author regards the urine as containing very small quantities of some pyridine bases which are analogous or identical with those obtained by **Gautier** and **Etard** from decomposing fish.

The distinguished Italian toxicologist **Selmi** was, perhaps, the first to draw attention to the probable formation of basic substances in the living body during those pathological changes brought on by the presence of pathogenic germs; and in a memoir presented to the Academy of Sciences of Bologna, in December, 1880, he announced that infectious diseases, or those in which there occurs an internal disarrangement of some element, either plasmic or histological, must be accompanied or followed by an elimination of more or less characteristic products, which would be a sign of the pathological condition of the patient. To support this theory he examined a number of pathological urines, and succeeded in obtaining from them basic substances, some of which were poisonous, others not. Thus, a specimen of urine from a patient with progressive paralysis gave two bases strongly resembling nicotine and coniine; from other pathological urines the bases obtained usually had either an ammoniacal or trimethylamine odor. A strong confirmation of SELMI's theory is seen in the observations made by BOUCHARD, VILLIERS, LEPINE, GAUTIER, and others, all of whom have found basic substances in the urine of various diseases.

It is now a well-established fact that the urine of disease, as cholera (BOUCHARD) and septicæmia (FELTZ), etc., is far more poisonous than normal urine. That poisons which are generated within the body by the activity of bacteria can be excreted in the urine is seen in the fact that immunity to the action of bacillus pyocyaneus has been conferred on animals by previous injection of urine taken from animals inoculated with that bacillus (BOUCHARD) or with filtered cultures of the same (CHARRIN and RUFFER).

Unfortunately, none of these bases supposedly characteristic of pathological urines have been isolated in a chemically pure condition; nor has the study of normal urine been carried sufficiently far to show the positive absence of such bodies.

VILLIERS has denied the existence of alkaloids in normal urine, and this has been confirmed experimentally by STADTHAGEN, who, moreover, agreed with FELTZ and RITTER that specific organic poisons are absent from normal urine. The observed physiological action is therefore largely (70–80 per cent.), or wholly, due to the potassium salts present.

Leucomaïnes of the Saliva.

According to the statement of GAUTIER (1881), normal human saliva contains divers toxic substances in small quantities which differ very much in their action according to the time of their secretion, and probably according to the individual gland in which they are secreted. The aqueous extract of saliva at 100° is poisonous or narcotic in its action toward birds. To show the presence of basic substances, the aqueous extract was slightly acidulated with dilute hydrochloric acid, then precipitated by MAYER's reagent; the precipitate was washed, then decomposed by hydrogen sulphide, and the solution filtered. The filtrate on evaporation gave a residue consisting of microscopic slender needles of a soluble hydrochloride. This salt, purified by extraction with absolute alcohol, forms soluble crystalline, but easily decomposable double salts with platinum chloride and with gold chloride. The solution of the hydrochloride produces an immediate precipitate of Prussian blue in a mixture of potassium ferricyanide and ferric chloride, and when injected into birds produces a condition of stupor.

Leucomaïnes from other Tissues of the Body.

SELMI's work upon the formation of ptomaïnes during the process of putrefaction led many investigators to doubt the production of these bases by the decomposition of the proteid or other complex molecules. To substantiate this, a number of chemists, especially Italian, endeavored to show that SELMI's bases, to a large extent at least, exist preformed in the various tissues. PATERNÒ and SPICA (1882) succeeded in extracting from fresh blood as well as from fresh albumin of eggs substances identical, or at least similar, to those designated under the name of ptomaïnes. Their observations, however, were confined to the detection of alkaloidal reactions in the various extracts obtained by DRAGENDORFF's method, and at no time were they in possession of a definite chemical individual. MARINO-

ZUCO (1885) was more successful, inasmuch as he succeeded in obtaining from fresh tissues and organs relevant quantities of a base identical with choline, and, in addition, he obtained extremely minute traces of other alkaloidal bodies. One of these, obtained by the STAS method from the liver and spleen of an ox, exhibited in hydrochloric acid solution a beautiful violet fluorescence resembling very much that of the salts of quinine. A similar base, probably identical with this one, was obtained by BENCE JONES and DUPRÉ (1856) from liver, nerves, tissues, and other organs, and was named by them "animal chinoidine." A greenish-blue fluorescence is frequently observable in the alcoholic extracts of decomposing glue as well as from other putrefying substances. From a number of very thorough experiments, he concluded that basic substances do not preëxist in fresh organs, but that the acids employed in the process of extraction exert a decomposing action upon the lecithin present in the tissues, resulting in the formation of choline. He further showed that the method of DRAGENDORFF, on account of the larger quantity of extractives which form, invariably gave a larger yield of this base than did the STAS-OTTO method. Similar observations were made by GUARESCHI and MOSSO, by COPPOLA and others. At the present time there is no doubt that some basic substances, among these choline, can be formed by the action of reagents, and, on the other hand, it is equally well demonstrated that similar bases do preëxist in the physiological condition of the tissues and fluids of the body.

Recently R. WURTZ has obtained from normal blood a number of crystalline products of alkaline reaction, which form well-crystallizable double salts with gold, platinum, and mercuric chlorides. These, however, have not been as yet subjected to analysis, because of the minute quantities which were isolated.

MORELLE (1886) showed the presence in the spleen of the ox of a base, the hydrochloride of which crystallized in deliquescent needles and likewise formed crystalline platino- and aurochlorides. From experiments made by LABORDE, the base would seem to possess decided toxic properties, bringing on a dyspnœic condition with convulsive movements and loss of motion. The post-mortem examinations revealed an extended visceral œdematous infiltration, and stoppage of the heart in systole.

A. W. Blyth has claimed to have isolated from milk two alkaloidal substances, namely galactine, the lead salt of which is said to have the formula $Pb_2O_3C_{54}H_{18}N_4O_{25}$, and lactochrome, the mercury salt of which is represented by the formula $HgOC_6H_{18}NO_6$.

Leucomaïnes of the Venoms of Poisonous Serpents.

The study of the chemistry of the venoms of serpents and of batrachians is fraught with so many difficulties and with so much danger, that we cannot wonder at the present unsatisfactory condition of our knowledge in regard to the poisonous principles which they contain. Much of the work that has been done hitherto is not only inaccurate and very contradictory, but is far from meeting the requirements of exact toxicological research. From recent investigations it seems, however, to be quite certain that the most active constituent of the venom of serpents is not alkaloidal in its nature as has been supposed by some. In 1881 Gautier announced the isolation of two alkaloids from the venom of the cobra which gave precipitates with tannin, Mayer's reagent, Nessler's reagent, iodine in potassium iodide, etc. They formed crystallizable platinochlorides and aurochlorides, and also crystalline, neutral, somewhat deliquescent hydrochlorides. The neutral or slightly acid solutions produced an immediate precipitate of Prussian blue in a mixture of potassium ferricyanide and ferric chloride. These substances possess a decided physiological action, though Gautier himself does not consider them to be the most dangerous constituents of the venoms. This observation of Gautier as to the presence of distinct basic substances in venoms is at variance with that of Wolcott Gibbs, who has been unable to obtain an alkaloid from the rattlesnake (Crotalus) venom. S. Weir Mitchell and E. T. Reichert likewise state that they have been utterly unable to substantiate GAUTIER's statements. Still more recently WOLFENDEN, in an elaborate paper on the nature of cobra venom, has confirmed WOLCOTT GIBBS as to the entire absence of any alkaloidal body.

MITCHELL and REICHERT have made a careful study of the venoms of various serpents, such as cobra, rattlesnake, moccasin, and Indian viper, and have succeeded in isolating two proteid constituents, one belonging to the class of globulins and the other to the peptones. The peptone is said to be non-precipitable by alcohol. According to them, the globulin constituent consists of at least three distinct globulins. They found that boiling coagulates and destroys the globulin as a poison, but leaves the venom peptone toxically unchanged, so that the solution, though still poisonous, fails to produce the characteristic local lesions due to fresh or unboiled venom. On the other hand, GAUTIER asserts that the venom is not sensibly altered on being heated to 120°–125°, and that the toxic action remains constant even when all the proteid constituents are removed, thus showing that the toxic action cannot be attributed to the albuminoids. The venom peptone from the rattlesnake or the moccasin, however, when injected into animals produced toxic effects which were marked by an œdematous swelling over the site of injection; the tumor was filled with serum, and so also was the subcutaneous cellular tissue. Furthermore, a gradual breaking down of the tissues occurred, accompanied by rapid putrefactive changes and a more or less extensive slough. That peptones may possess intensely poisonous properties has been shown to be the case by a number of authors, among whom may be mentioned SCHMIDT-MÜLHEIM, HOFMEISTER, POLLITZER, and others. BRIEGER has, moreover, demonstrated that the formation of peptones in the process of digestion is accompanied by the development of a toxic ptomaïne which he has named peptotoxine.

The venom globulins, on the other hand, though present in less quantity than the peptones, induced the same remarkable local effects seen on injection of the pure venom.

They cause local bleedings, destroy the coagulability of the blood, and rapidly corrode the capillaries.

These results of MITCHELL and REICHERT, which are given here somewhat in full, have been questioned by WOLFENDEN, who, while agreeing in the main that the poisonous property of venom is due to proteid constituents, regards their peptone not as a true peptone, but rather as one or more bodies of the albumose group of proteids. He likewise regards the globulin of moccasin venom to be some other proteid body. According to him, the cobra venom owes its toxicity to the proteids, globulin, serum-albumin, acid albumin. Occasionally there seem to be present traces of peptone and of hemialbumose.

BRIEGER was at first apparently inclined to believe that the action of venoms is due to animal alkaloids, on the ground that these bases are extremely soluble, and hence always go into solution along with the likewise very soluble proteid constituents, and that the difficulty in their isolation lies in the elimination of these proteids. Since then BRIEGER and FRÄNKEL pointed out the poisonous nature of some bacterial proteids, and also showed that cobra poison yields with alcohol a precipitate which gives proteid reactions.

The proteids of serpents' venom should be compared with the poisonous proteids formed by the activity of the pathogenic bacteria, as well as with similar compounds, the *phytalbumoses* of castor seeds, jequirity, etc. Possibly similar compounds will be found in croton and other species of ricinus, jatropha, loco-weed, etc. The poisons secreted by certain spiders and fish may be mentioned in this connection.

CLOËZ and GRATIOLET in 1852 examined the poison contained in the cutaneous pustules of some batrachians, and succeeded in extracting a substance which gave a white precipitate with mercuric chloride and formed a platinum double salt. Beyond this meagre information very little is known in regard to the character of these poisons, though ZALESKY, in 1866, announced the isolation of an alkaloid to which he assigned the formula $C_{34}H_{60}N_2O_5$, and which he named salamandarine. According to DUTARTRE (1890) this base is a leucomaïne. and similar products, but with different physiological action, are to be found in other batrachians, as the toad, triton(?), green and red frogs, and in the epidermis of some fish. According to CALMEIL, the poison from the toad contains methyl-carbylamine and isocyanacetic acid.

TABLE OF LEUCOMAÏNES.

Formula.	Name.	Discoverer.	Source.	Physiological action.
$C_5 H_5 N_5$	Adenine.	Kossel.	Nuclein-containing organs.	Non-poisonous; muscle stimulant.
$C_5 H_4 N_4 O$	Hypoxanthine.	Scherer.	Nuclein-containing organs.	Non-poisonous; muscle stimulant.
$C_5 H_5 N_5 O$	Guanine.	Unger.	Nuclein-containing organs, guano.	Non-poisonous; muscle stimulant.
$C_5 H_4 N_4 O_2$	Xanthine.	Marcet.	Nuclein-containing organs, calculi.	Non-poisonous; muscle stimulant.
$C_6 H_6 N_4 O_2$	Heteroxanthine.	Salomon.	Urine.	
$C_7 H_8 N_4 O_2$	Paraxanthine.	Thudichum Salomon.	"	Poisonous.
$C_7 H_8 N_4 O_3$	Carnine.	Weidel.	Liebig's meat extract.	Non-poisonous; muscle stimulant.
$C_4 H_5 N_5 O$	Pseudoxanthine(?)	Gautier.	Muscle.	
$C_5 H_{14} N_2$	Gerontine.	Grandis.	Liver of dogs.	Poisonous.
$C_2 H_5 N$ (?)	Spermine.	Schreiner.	Sperma, in tissues of leucocythæmics.	Non-poisonous.
$C_5 H_8 N_4 O$	Cruso-creatinine.	Gautier.	Muscle.	
$C_5 H_{10} N_4 O$	Xantho-creatinine	"	"	Poisonous.
$C_9 H_{19} N_7 O_4$	Amphi-creatine.	"	"	
$C_{11} H_{24} N_{10} O_5$	Unnamed.	"	"	
$C_{12} H_{25} N_{11} O_5$	"	"	"	
$C_7 H_{12} N_4 O_2$	"	Pouchet.	Urine.	
$C_3 H_5 NO_2$	"	"	"	
$C_{24} H_{60} N_2 O_5$	Salamandarine.	Zalesky.	Salamander.	Poisonous.

CHAPTER XIII.

THE AUTOGENOUS DISEASES.

ALL living things are composed of cells. The simplest forms of life are unicellular, and in these all the functions of life devolve upon the single cell. Absorption, secretion, and excretion must be carried on by the same cell. A collection of unicellular organisms might be compared to a community of men with every individual his own tailor, shoemaker, carpenter, cook, farmer, gardener, blacksmith, etc. However, only the lowest forms of life are unicellular; all others are multicellular. In the higher animals there is a differentiation not only in the size and structure of the cells, but in the labor which they perform. The body of man may be compared to a community in which labor has been specialized. Certain groups of cells, which we designate by the term "organ," take upon themselves the task of doing some special line of work, the well-doing of which is essential to the health, not only of that group, but of other groups as well, or of the body as a whole. There is an interdependence among the various organs. Certain groups of cells supply the fluids or juices which act as digestants, and among these there is again a division of labor. The salivary glands supply a fluid which partially digests the starch of our food; the peptic glands supply the gastric juice which does the preliminary work in the digestion of the proteids; while the pancreatic juice completes the digestion of the starches begun in the mouth, of the proteids begun in the stomach, and does the special work of emulsifying the fats. But even some of these products of complete digestion would be harmful should they enter the circulation unchanged. The peptones must be converted into serum-albumin by the absorbing mechanism of the walls of the intestines, and while 10 per cent.

of the fat of the food is split up into glycerin and fatty acids by the action of the pancreatic juice, a much smaller per cent. enters the thoracic duct in this divided form. The food may be taken in proper quality and quantity; the digestive juices may do their work promptly and properly, but if the absorbents fail to perform their functions properly, disease results. It may happen that the failure lies in improper or imperfect assimilation and the result becomes equally disastrous, and with the effects of non-elimination we are fairly conversant. Of the myriads of cells in the healthy human body there are none which are superfluous. It is true that among these ultimate entities of existence, death is constantly occurring, but in health regeneration goes on with equal rapidity and each organ continues to do its daily and hourly task. The microscope has made us familiar with the size and shape of the various cells of the body, and students of pathology have described the alterations in form and size characteristic of various disease states. But we must remember that in the study of these ultimate elements of life there are other things, besides their morphological history, to investigate. They are endowed with life, and they, as well as the germs, have a physiology and chemistry which we know but slightly. They are influenced beneficially or harmfully, as the case may be, by their environment. They grow and perform their functions properly when supplied with the needed pabulum. They are not immune to poisonous agents. They are injured when the products of their own activity accumulate about them.

The object in writing this chapter has been to collect what evidence we may concerning those diseases which arise from imperfect or improper activity of the cells of the body, not due to the introduction of foreign cells. To designate this class of diseases we have selected the word *autogenous*, and we understand that in these diseases the *materies morbi* is a product of some cell of the body, and not, as in the case of the infectious diseases, of cells introduced from without the body.

It is true, without exception so far as we know, that the excretions of all living things, plants and animals, contain substances which are poisonous to the organisms which excrete them. A man may drink only chemically pure water, eat only that food which is free from all adulterations, and breathe nothing but the purest air, free from all organic matter, both living and dead, and yet that man's excretions would contain poisons. Where do these poisons originate? They are formed within the body. They originate in the metabolic changes by which the complex organic molecule is split up into simpler compounds. We may suppose—indeed, we have good reasons for believing—that the proteid molecule has certain lines of cleavage along which it breaks when certain forces are applied, and that the resulting fragments have also lines of cleavage along which they break under certain influences, and so on until the end-products, urea, ammonia, water, and carbon-dioxide are reached; also that some of these intermediate products are highly poisonous has been abundantly demonstrated. The fact that the hydrocyanic acid molecule is a frequent constituent of the leucomaïnes is one of great significance. We know that chemical composition is an indication of physiological action, and the intensely poisonous character of some of the leucomaïnes conforms to this fact. It matters not whether the proteid molecule be broken up by organized ferments, bacteria, or by the unorganized ferments of the digestive juices, by the cells of the liver or by those still unknown agencies, which induce metabolic changes in all the tissues—in all cases poisons may be formed. These poisons will differ in quality and quantity according to the proteid which is acted upon, and according to the force which acts.

Peptones formed during digestion do not in health reach the general circulation. When injected directly into the blood they act as powerful poisons. They destroy the coagulability of the blood, lower blood-pressure, and in large quantities cause speedy death. **Brunton** attributes the lassitude, depression, sense of weight in the limbs, and dulness in the head occurring in the well-fed, inactive man, after his meals, to poisoning with peptones. The remedy which he proposes is less food, especially less nitrogenous food, and more exercise. That some substance resulting from the proteids of the food is the cause of this trouble BRUNTON thinks is evidenced by the fact that the weakness and languor are apparently less after meals consisting of farinaceous foods only.

That peptone finds its way into the general circulation frequently is shown by its detection in the urine in many diseased conditions, some of which are infectious and others autogenous in character. However, propeptonuria, or albumosuria, is more common than peptonuria, and we have already seen that many of the bacterial albumoses are among the most highly poisonous bodies known, but the action of the albumoses formed during digestion has not, so far as we know, been studied. The valuable work of KÜHNE and CHITTENDEN on the chemical character of these bodies should be supplemented by a thorough investigation of their physiological effects when injected into the blood. It is more than probable that valuable information would be secured by such studies. That albumose is frequently found in the urine is shown by the following list of diseases in which it has been observed, given in the last edition of the work of NEUBAUER and VOGEL on the urine: KÖSNER has found it in spermatorrhœa; KÖPPEN, in mental diseases without spermatorrhœa; KAHLER, in osteomalacia; BENCE JONES, in multiple myeloma; SENATOR and others, in dermatitis, intestinal ulcer, liver abscess, croupous pneumonia, apoplexy, vitium cordis, resectio coxæ, parametritis, endocarditis, typhoid fever, nephritis, phthisis, etc.; LOEB, in measles and scarlet fever; LEUBE, in urticaria; and LASSAR, after inunctions of petroleum. KÖTTNITZ, FURSTNER, and others, find albumose frequently in the urine in mental diseases. Evidently, there is much to learn from the study of the conditions accompanied by the elimination of the albumoses in the urine. It is more than probable that the acute Bright's disease following scarlet fever, diphtheria, and the other acute infectious diseases, owes its existence to the poisonous albumoses of these diseases. PRIOR has recently shown that undigested egg albumin is sometimes absorbed and produces marked disturbances. A boy, after eating sixteen raw eggs, had a high fever accompanied by the appearance of both albumin and hæmoglobin in the urine.

BRIEGER obtained by digesting fibrin with gastric juice a substance which gives reactions with many of the general alkaloidal reagents and to which he has given the name "peptotoxine." A few drops of a dilute aqueous solution of this substance sufficed to kill frogs within fifteen minutes. The frogs became apparently paralyzed and did not respond to stimuli. Slight tremor was perceptible in the muscles of the extremities. Rabbits of about one kilogramme weight were given from 0.5 to 1 gramme of the extract subcutaneously. About fifteen minutes after the injection, paralysis beginning in the posterior extremities set in; the animal fell into a somnolent condition, sank and died. In some rabbits several hours elapsed before the above-mentioned symptoms appeared.

Peptotoxine was found by BRIEGER to be formed not only by the digestive juice, but to be among the first putrefactive products of proteids, as fibrin, casein, brain substance, liver, and muscle.

It is highly probable that many of the nervous symptoms which accompany some forms of dyspepsia are due to the formation and absorption of poisonous substances.

In some persons the tendency to the formation of poisons out of certain foods is very marked. Thus, there are some to whom the smallest bit of egg is highly poisonous; with others, milk will not agree; and instances of this kind are sufficiently numerous to give rise to the adage, "What is one man's meat is another man's poison."

BRUNTON is of the opinion that the condition which we term "biliousness," and which is most likely to exist in those who eat largely of proteids, is due to the formation of poisonous alkaloids; but of this we have no positive proof.

Whether or not the unorganized digestive ferments ever find their way into the blood in quantity sufficient to cause deviations from health, we are not in a position to state definitely. The older physiological chemists teach us that pepsin and trypsin are frequent, if not constant constituents of normal urine, but their experiments were made without any reference to the possibility of the ferments which they found being formed by the bacteria of the urine, and after carefully going over the literature of the subject we are not prepared to pass judgment on the truth of their statements. However this may be, the fact that these ferments manifest a marked toxicological effect when introduced into the blood is of great interest, especially at this time. HILDEBRANDT has recently reported the results of some experiments made by himself upon this subject. He finds that a fatal dose of pepsin for dogs is from 0.1 to 0.2 gramme per kilogramme of body weight. The subcutaneous injection of these quantities is followed by a marked elevation of temperature, which he designates as "ferment fever." This fever begins within an hour after the injection, reaches its maximum after from four to six hours, and may continue for some days. On the day preceding death, the temperature generally falls below the normal. During the period of elevation there are frequent chills.

The symptoms which accompanying the fever vary somewhat with the species of animal. Rabbits lose flesh nothwithstanding the fact that they continue for a while to eat well, they become very weak, and death is preceded by convulsive movements. Dogs tremble in the limbs, become uncertain in gait, and vomiting, dyspnœa and coma are followed by death.

On section there is observed parenchymatous degeneration of the muscles of the heart and similar changes in the liver and kidney. There are abundant hemorrhages in the intestinal canal, in PEYER'S patches, in the mesenteric glands; and in the lungs in cats. Thrombi are frequently found in the lungs and in some cases in the kidneys.

The effect upon the coagulability of the blood is worthy of note. At first there is a period during which the coagulability of the blood is greatly lessened, then follows a period of greater rapidity in coagulating, and it is in this latter stage that the thrombi are formed.

These experiments are interesting not only as a possible explanation of the cause of some of the autogenous fevers, which will be discussed later, but in view of the present tendency to inject such complex animal solutions as BROWN-SÉQUARD's elixir and KOCH's lymph subcutaneously, and they will probably cause us to exercise a little more care in this direction.

That certain febrile conditions are autogenous there can be no doubt. These, like other diseases originating within the system, may be due to either of the following causes: 1. There may be an excessive formation of poisonous substances in the body. Thus, BOUCHARD has shown that the urine excreted during the hours of activity is much more poisonous than that excreted during the hours of rest. Both physical and mental labor are accompanied by the formation of these deleterious bodies, and if the hours of labor are prolonged and those of rest shortened, there will be an accumulation of effete matters within the system. 2. The accumulation of the poisonous matters may be due to deficient elimination. 3. Some organ whose duty it is to change harmful into harmless bodies may fail to properly perform its functions. Illustrations of diseased conditions arising from these several causes will be given.

First, we may mention fatigue fever, which is by no means uncommon, and from which the overworked physician not infrequently suffers. One works night and day for some time; elimination seems to proceed normally; but after a few days there is an elevation of temperature of from one to three degrees, the appetite is impaired, and then if the opportunity for rest is at hand sound and restful sleep is impossible. The tired man retires to his bed expecting to fall asleep immediately, but he tosses from side to side all night, or his sleep is fitful and unrefreshing. The brain is excited and refuses to be at rest. The senses are alert, and all efforts to sink them in repose are unavailing. Fatigue fever is frequently observed in armies upon forced marches, especially if the troops are young and unaccustomed to service. MOSSO has studied this fever in the Italian army. He states that in fatigue the blood is subjected to a process of decomposition brought about by the infiltration into it from the tissues of poisonous substances, which, when injected into the circulation of healthy animals, induce malaise and all the signs of excessive exhaustion. It is possible that in this decomposition of the blood the fibrin-ferment, which, according to SCHMIDT, is held in combination in the colorless corpuscles, is liberated; and it has been shown by EDELBERG that the injection of small quantities of free fibrin-ferment into the blood causes fever, while the injection of larger quantities is followed by the formation of thrombi, as has been demonstrated by the experiments of EDELBERG, BONNE, BIRK, and KÖHLAR.

Fatigue fever is often accompanied, especially during the period of elevation, by chilly sensations, and consequently it is pronounced malarial and quinine is administered, but it does no good—often harm, by increasing cerebral excitement. The proper treatment is prolonged rest, with proper attention to elimination.

Then there is the fever of exhaustion, which differs from fatigue fever only in degree. It is brought on by prolonged exertion without sufficient rest and often without sufficient food. The healthy balance between the formation and elimination of effete matter is disturbed, and it may be weeks before it is reëstablished—indeed, it may never be regained, for some of those cases terminate fatally. The fever of exhaustion may take on the typhus form, delirium may appear, muscular control of the bowels may be lost, and death may result.

That the fever of exhaustion has been mistaken for typhoid by some of the ablest clinical teachers is shown by PETER in the following quotation. "It was in 1852," says he, "when entering upon my clinical studies and ardent in my attendance at the clinic of CHOMEL, I was witness of the following instance: A young man was received under the celebrated professor's charge suffering from prostration, muscular pain, and rhachialgia. CHOMEL made the examination with all the care and attention used by him; then

—as was also usual with him in the presence of the patient —he gave the diagnosis in Latin, which was '*Aut febris peyerica, aut variola incipientis*' (either typhoid fever or incipient smallpox). I felt rather dissatisfied at a diagnosis so little precise by one so eminent in his art. The truth of the matter was, though CHOMEL was not aware of it, this young fellow in a state of destitution had walked from Compiègne to Paris, sleeping by the wayside at night and nourishing himself with such refuse food as chance supplied. It was under such circumstances the patient had developed febrile symptoms. The day after his admission, and simply from rest in bed, he felt better, and the day following he was altogether well."

That all cases of the fever of exhaustion do not terminate so rapidly as that instanced above many physicians know. We have seen at least one such case terminate fatally.

Then, again, there is the fever of non-elimination, which all physicians of experience have observed. There is a feeling of languor, the head aches, the tongue is coated, the breath offensive, and the bowels constipated. The physician fears typhoid fever, but finds that a good, brisk cathartic dissipates all unpleasant symptoms, and the temperature falls to the normal. This fever is also liable to appear among those who are confined to bed from other causes. BRUNTON says: "No one who has watched cases of acute diseases, such as pneumonia, can have failed to see how a rise of temperature sometimes coincides with the occurrence of constipation, and is removed by opening the bowels." The surgeon and obstetrician have often had cause to rejoice when they have found a fever, which they feared indicated septicæmia, disappearing after free purgation.

BOUCHARD has shown that normal feces contain a highly poisonous substance, which may be separated from them by dialysis, and which, when administered to rabbits, produces violent convulsions. He estimates that the amount of poisonous alkaloids formed in the intestines of a healthy man each twenty-four hours would be quite sufficient to kill, if it was all absorbed. He proposes the term "stercoræmia" for that condition which results from arrest of excretion from the intestine.

It is more than probable that the poisons of the intestines are due to the bacteria which are normally present; but this would not exclude the fever of non-elimination from the list of autogenous diseases. The bacterial cells which are normally present in the intestines cannot be regarded as invaders from without.

It would seem from some recent studies that not all surgical fevers are due to bacterial activity. The absorption of aseptic blood-clots and of disintegrated tissue in cases of complicated fractures and contusions of the joints is accompanied by an elevation of the temperature above normal. A like result may follow the intravenous injection of a sterile solution of hæmoglobin or of the blood of another animal. The causative agent in the production of these fevers remains unknown. In the blood of twelve out of fifteen patients with aseptic fever, at the clinic of NOTHNAGEL, HAMMERSCHLAG has found free fibrin-ferment, but in five persons without fever he found the same substance in the blood. This leaves the causative agent in the production of the aseptic, or, more properly speaking, the non-bacterial, fevers unknown.

The chemical theory of so-called uræmia has received support in recent researches, notwithstanding the fact that the old idea that urea is the active poison and the theory of FRERICHS that ammonium carbonate is the active agent have been abandoned.

LANDOIS laid bare the surface of the brain in dogs and rabbits, and sprinkled the motor area with creatine, creatinine, and other constituents of the urine. Urea, ammonium carbonate, sodium chloride, and potassium chloride had but slight effect; but creatine, creatinine, and acid sodium phosphate caused clonic convulsions on the opposite side of the body which later became bilateral. The convulsions continued at intervals for from two to three days, when, growing gradually weaker, they disappeared. LANDOIS concludes that chorea gravidarum is a forerunner of eclampsia. These experiments have been confirmed by LEUBUSCHER and ZEICHEN.

FALCK injected into both sound and nephrotomized animals fresh urine, urine and the ferment of MUSCULUS and LEA, and urine which had undergone spontaneous decomposition, without producing any symptoms which were comparable with those observed in uræmia. However, he did find that if a few drops of an infusion of putrid flesh were added to the urine before injection all the typical symptoms of uræmia were induced. That the infusion of putrid flesh alone had no effect was also demonstrated. This would lead us to believe that some ferment in the infusion converts some constituent of the urine into a highly poisonous body. In this connection attention may be called to the fact that creatine may be converted by the action of certain germs into methyl-guanidine, which produces convulsions. Whether such conversion occurs in uræmia or not, and if it does what the cause of it is, are questions which must be left for future investigations to decide. It would be well for someone to test the brain and blood of a person who has died in uræmic convulsions for methyl-guanidine.

That there is a marked disturbance of tissue metabolism caused by the inhalation of vitiated air has been shown by ARAKI. In the urine of animals rendered unconscious by being kept in a confined space this experimenter found albumin, sugar, and lactic acid. If the animals had been kept without food for some days before being subjected to this experiment albumin and lactic acid were found, but no sugar appeared. This was undoubtedly due to the fact that the glycogen of the body had been exhausted by the fasting. Identical results were observed in animals which were poisoned with carbon monoxide. Dogs which were poisoned with curare, and in which the respiratory movements were maintained artificially, secreted very little urine; but the blood was found to contain considerable quantities of sugar and lactic acid. The urine of frogs in which the respiration was retarded by the production of tetanus with strychnine secreted urine containing sugar and lactic acid. In the urine of three epileptics there were found albumin and lactic acid directly after the seizure. The factor common to all these cases is diminished oxygenation of the blood, and to this is ascribed the appearance of the abnormal constituents of the urine. These investigations demonstrate the influence of impure air upon the chemistry of the living cells of the animal body.

CHAPTER XIV.

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